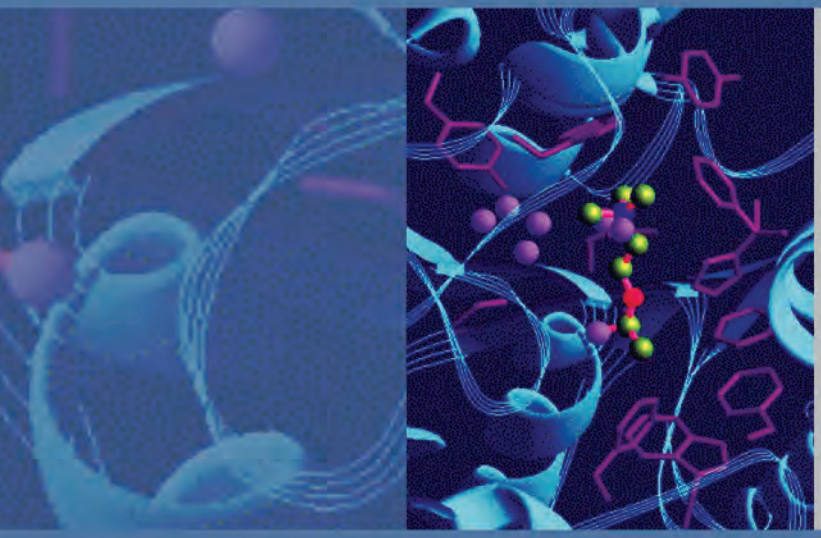

# Surveyor Plus

## Getting Started with ChromQuest 5.0 Tutorial

60053-97125 Revision B January 2009

DOCUMENTATION SURVEY

Thermo SCIENTIFIC

Release history: Revision A released March 2008. Revision B released January 2009.

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# Regulatory Compliance

Thermo Fisher Scientific performs complete testing and evaluation of its products to ensure full compliance with applicable domestic and international regulations. When the system is delivered to you, it meets all pertinent electromagnetic compatibility (EMC) and safety standards as described in the next section or sections by product name.

Changes that you make to your system might void compliance with one or more of these EMC and safety standards. Changes to your system include replacing a part or adding components, options, or peripherals not specifically authorized and qualified by Thermo Fisher Scientific. To ensure continued compliance with EMC and safety standards, replacement parts and additional components, options, and peripherals must be ordered from Thermo Fisher Scientific or one of its authorized representatives.

This section contains regulatory compliance information for the following devices of the Surveyor Plus family of LC instruments:



# Surveyor LC Pump Plus

## WEMC Directive 89/336/EEC amended by 92/31/EEC and 93/68/EEC

EMC compliance has been evaluated by Underwriters Laboratories Inc.

| | | | |
|---|---|---|---|
| EN 55011 | 1998 | EN 61000-4-3 | 2002 |
| EN 61000-3-2 | 1995, A1; 1998, A2; 1998, A14; 2000 | EN 61000-4-4 | 1995, A1; 2001, A2; 2001 |
| IEC 61000-3-2 | 2000 | EN 61000-4-5 | 1995, A1; 2001 |
| EN 61000-3-3 | 1995 | EN 61000-4-6 | 1996, A1; 2001 |
| IEC 61000-3-3 | 1994 | EN 61000-4-11 | 1994, A1; 2001 |
| EN 61326-1 | 1997 | | |
| EN 61000-4-2 | 1995 A1; 1998 A2; 2001 | CISPR 11 | 1999, A1; 1999, A2; 2002 |

FCC Class A, CFR 47 Part 15 Subpart B: 2004

## Low Voltage Safety Compliance

Low voltage safety compliance has been evaluated by TUV Rheinland of North America, Inc. This device complies with Low Voltage Directive 73/23/EEC and harmonized standard EN 61010-1:2001.

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# Surveyor Autosampler Plus

## EMC Directive 89/336/EEC amended by 92/31/EEC and 93/68/EEC

EMC compliance has been evaluated by Underwriters Laboratories Inc.

| | | | |
|---|---|---|---|
| EN 55011 | 1998 | EN 61000-4-3 | 2002 |
| EN 61000-3-2 | 1995, A1; 1998, A2; 1998, A14; 2000 | EN 61000-4-4 | 1995, A1; 2001, A2; 2001 |
| IEC 61000-3-2 | 2000 | EN 61000-4-5 | 1995, A1; 2001 |
| EN 61000-3-3 | 1995 | EN 61000-4-6 | 1996, A1; 2001 |
| IEC 61000-3-3 | 1994 | EN 61000-4-11 | 1994, A1; 2001 |
| EN 61326-1 | 1997 | | |
| EN 61000-4-2 | 1995 A1; 1998 A2; 2001 | CISPR 11 | 1999, A1; 1999, A2; 2002 |
| FCC Class A, CFR 47 Part 15 Subpart B: 2004 | | | |

## Low Voltage Safety Compliance

Low voltage safety compliance has been evaluated by TUV Rheinland of North America, Inc. This device complies with Low Voltage Directive 73/23/EEC and harmonized standard EN 61010-1:2001.

# Surveyor PDA Plus Detector

## EMC Directive 89/336/EEC amended by 92/31/EEC and 93/68/EEC

EMC compliance has been evaluated by Underwriters Laboratories Inc.

| | | | |
|---|---|---|---|
| EN 55011 | 1998 | EN 61000-4-3 | 2002 |
| EN 61000-3-2 | 1995, A1; 1998, A2; 1998, A14; 2000 | EN 61000-4-4 | 1995, A1; 2001, A2; 2001 |
| IEC 61000-3-2 | 2000 | EN 61000-4-5 | 1995, A1; 2001 |
| EN 61000-3-3 | 1995 | EN 61000-4-6 | 1996, A1; 2001 |
| IEC 61000-3-3 | 1994 | EN 61000-4-11 | 1994, A1; 2001 |
| EN 61326-1 | 1997 | | |
| EN 61000-4-2 | 1995 A1; 1998 A2; 2001 | CISPR 11 | 1999, A1; 1999, A2; 2002 |
| FCC Class A, CFR 47 Part 15 Subpart B: 2003 | | | |

## Low Voltage Safety Compliance

Low voltage safety compliance has been evaluated by TUV Rheinland of North America, Inc.This device complies with Low Voltage Directive 73/23/EEC and harmonized standard EN 61010-1:2001.

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# Surveyor UV/Vis Plus Detector

## EMC Directive 89/336/EEC amended by 92/31/EEC and 93/68/EEC

EMC compliance has been evaluated by Underwriters Laboratories Inc.

| | | | |
|---|---|---|---|
| EN 55011 | 1998 | EN 61000-4-3 | 2002 |
| EN 61000-3-2 | 1995, A1; 1998, A2; 1998, A14; 2000 | EN 61000-4-4 | 1995, A1; 2001, A2; 2001 |
| IEC 61000-3-2 | 2000 | EN 61000-4-5 | 1995, A1; 2001 |
| EN 61000-3-3 | 1995 | EN 61000-4-6 | 1996, A1; 2001 |
| IEC 61000-3-3 | 1994 | EN 61000-4-11 | 1994, A1; 2001 |
| EN 61326-1 | 1997 | | |
| EN 61000-4-2 | 1995 A1; 1998 A2; 2001 | CISPR 11 | 1999, A1; 1999, A2; 2002 |

FCC Class A, CFR 47 Part 15 Subpart B: 2004

## Low Voltage Safety Compliance

Low voltage safety compliance has been evaluated by TUV Rheinland of North America, Inc.This device complies with Low Voltage Directive 73/23/EEC and harmonized standard EN 61010-1:2001.

# Surveyor FL Plus Detector

## EMC Directive 89/336/EEC, 92/31/EEC, 93/68/EEC

EMC compliance has been evaluated by TUV Rheinland of North America, Inc.

| | | | |
|---|---|---|---|
| EN 61326-1 | 1997; A1, 1998; A2, 2001; A3, 2003 | EN 61000-4-4 | 1995, A1; 2001, A2; 2001 |
| EN 61000-3-2 | 2000 | EN 61000-4-5 | 2001 |
| EN 61000-3-3 | 1995; A1, 2001 | EN 61000-4-6 | 2003 |
| EN 61000-4-2 | 2001 | EN 61000-4-8 | 2001 |
| EN 61000-4-3 | 2002 | EN 61000-4-11 | 2001 |

FCC Class A, CFR 47 Part 15 Subpart B: 2005

## Low Voltage Safety Compliance

Low voltage safety compliance has been evaluated by TUV Rheinland of North America, Inc.

This device complies with Low Voltage Directive 73/23/EEC and harmonized standard EN 61010-1:2001, IEC 61010-1:2002, UL 61010 A-1:2004, CAN/CSA 22.2 61010-1:2004.

# Surveyor RI Plus Detector

## EMC Directive 89/336/EEC, 92/31/EEC, 93/68/EEC

EMC compliance has been evaluated by TUV Rheinland of North America, Inc.

| | | | |
|---|---|---|---|
| EN55011 | 1998; A1, 1999; A2, 2002 | EN 61000-4-3 | 2002 |
| CISPR11 | 1998 | EN 61000-4-4 | 1995, A1; 2001, A2; 2001 |
| EN 61326-1 | 1997; A1, 1998; A2, 2001; A3, 2003 | EN 61000-4-5 | 2001 |
| EN 61000-3-2 | 2000 | EN 61000-4-6 | 2003 |
| EN 61000-3-3 | 1995; A1, 2001 | EN 61000-4-11 | 2001 |
| EN 61000-4-2 | 2001 | | |

FCC Class A, CFR 47 Part 15 Subpart B: 2005

## Low Voltage Safety Compliance

Low voltage safety compliance has been evaluated by TUV Rheinland of North America, Inc.

This device complies with Low Voltage Directive 73/23/EEC and harmonized standard EN 61010-1:2001, IEC 61010-1:2002, UL 61010 A-1:2004, CAN/CSA 22.2 61010-1:2004.

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# FCC Compliance Statement

THIS DEVICE COMPLIES WITH PART 15 OF THE FCC RULES. OPERATION IS SUBJECT TO THE FOLLOWING TWO CONDITIONS: (1) THIS DEVICE MAY NOT CAUSE HARMFUL INTERFERENCE, AND (2) THIS DEVICE MUST ACCEPT ANY INTERFERENCE RECEIVED, INCLUDING INTERFERENCE THAT MAY CAUSE UNDESIRED OPERATION.

**CAUTION** Read and understand the various precautionary notes, signs, and symbols contained inside this manual pertaining to the safe use and operation of this product before using the device.

# Notice on Lifting and Handling of Thermo Scientific Instruments

For your safety, and in compliance with international regulations, the physical handling of this Thermo Fisher Scientific instrument ***requires a team effort*** to lift and/or move the instrument. This instrument is too heavy and/or bulky for one person alone to handle safely.

# Notice on the Proper Use of Thermo Scientific Instruments

In compliance with international regulations: Use of this instrument in a manner not specified by Thermo Fisher Scientific could impair any protection provided by the instrument.

# Notice on the Susceptibility to Electromagnetic Transmissions

Your instrument is designed to work in a controlled electromagnetic environment. Do not use radio frequency transmitters, such as mobile phones, in close proximity to the instrument.

For manufacturing location, see the label on the instrument.

# WEEE Compliance

This product is required to comply with the European Union's Waste Electrical & Electronic Equipment (WEEE) Directive 2002/96/EC. It is marked with the following symbol:

Thermo Fisher Scientific has contracted with one or more recycling or disposal companies in each European Union (EU) Member State, and these companies should dispose of or recycle this product. See www.thermo.com/WEEERoHS for further information on Thermo Fisher Scientific's compliance with these Directives and the recyclers in your country.

# WEEE Konformität

Dieses Produkt muss die EU Waste Electrical & Electronic Equipment (WEEE) Richtlinie 2002/96/EC erfüllen.  Das Produkt ist durch folgendes Symbol gekennzeichnet:

Thermo Fisher Scientific hat Vereinbarungen mit Verwertungs-/Entsorgungsfirmen in allen EU-Mitgliedsstaaten getroffen, damit dieses Produkt durch diese Firmen wiederverwertet oder entsorgt werden kann. Mehr Information über die Einhaltung dieser Anweisungen durch Thermo Fisher Scientific, über die Verwerter, und weitere Hinweise, die nützlich sind, um die Produkte zu identifizieren, die unter diese RoHS Anweisung fallen, finden sie unter www.thermo.com/WEEERoHS.

# Conformité DEEE

Ce produit doit être conforme à la directive européenne (2002/96/EC) des  Déchets d'Equipements Electriques et Electroniques (DEEE). Il est marqué par le symbole suivant:

Thermo Fisher Scientific s'est associé avec une ou plusieurs compagnies de recyclage dans chaque état membre de l’union européenne et ce produit devrait être collecté ou recyclé par celles-ci. Davantage d'informations sur la conformité de Thermo Fisher Scientific à ces directives, les recycleurs dans votre pays et les informations sur les produits Thermo Fisher Scientific qui peuvent aider la détection des substances sujettes à la directive RoHS sont disponibles sur www.thermo.com/WEEERoHS.

# Contents

# Preface

## About This Guide

This *Surveyor Plus Getting Started Guide with the ChromQuest 5.0 Chromatography Data System* manual contains an introduction to the modules of the Surveyor Plus LC system, as well as eleven tutorial chapters. The tutorials guide you through the process of setting up your system, creating data acquisition and shutdown methods, making your first injection, running multiple samples using a sequence table, and performing automated sample dilutions with a pretreatment method.

## Related Documentation

In addition to this guide, Thermo Fisher Scientific provides the following documents for the Surveyor Plus LC instruments and the ChromQuest 5.0 Chromatography Data System:

- *ChromQuest 5.0 Installation Guide*
- *ChromQuest 5.0 Administrator's Guide*
- *ChromQuest 5.0 User Guide*
- *ChromQuest 5.0 Reference Guide*
- *Surveyor Plus Preinstallation Requirements Guide*
- *Surveyor Plus Getting Connected Guide*
- *Surveyor Autosampler Plus Hardware Manual*
- *Surveyor LC Pump Plus Hardware Manual*
- *Surveyor UV/Vis Plus Detector Hardware Manual*
- *Surveyor PDA Plus Detector Hardware Manual*
- *Surveyor FL Plus Detector Hardware Manual*
- *Surveyor RI Plus Detector Hardware Manual*

The ChromQuest and Surveyor LC manuals are provided on the ChromQuest installation CD.

# Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

**CAUTION** Highlights hazards to humans, property, or the environment. Each CAUTION notice is accompanied by an appropriate CAUTION symbol.

**IMPORTANT** Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.

**Note** Highlights information of general interest.

**Tip** Highlights helpful information that can make a task easier.

# Contacting Us

There are several ways to contact Thermo Fisher Scientific for the information you need.

### ❖ To contact Technical Support

| | |
|---|---|
| Phone | 800-685-9535 |
| Fax | 561-688-8736 |
| E-mail | TechSupport.C+MS@thermofisher.com |
| Knowledge base | www.thermokb.com |

Find software updates and utilities to download at www.mssupport.thermo.com.

### ❖ To contact Customer Service for ordering information

| | |
|---|---|
| Phone | 800-532-4752 |
| Fax | 561-688-8731 |
| Web site | www.thermo.com/ms |

### ❖ To copy manuals from the Internet

Go to mssupport.thermo.com and click **Customer Manuals** in the left margin of the window.

### ❖ To suggest changes to documentation or to Help

- Complete a brief survey about this document by clicking the link below. Thank you in advance for your help.


- Send an e-mail message to the Technical Publications Editor at techpubs-lcms@thermofisher.com.

# Introduction to the Surveyor Plus LC System

The Surveyor Plus™ modular LC system and the ChromQuest™ data system are products of Thermo Scientific San Jose.

The Surveyor Plus modular LC system consists of the Surveyor LC Pump Plus, the Surveyor Autosampler Plus, and one or more of the following detectors: the Surveyor UV/Vis Plus Detector, the Surveyor PDA Plus Detector, the Surveyor FL Plus Detector, or the Surveyor RI Plus Detector.

This chapter contains a brief description of each module, as well as an overview of the system connections, the ChromQuest chromatography data system, and the tutorials provided in this manual.

**Contents**

# Surveyor PDA Plus Detector

The Surveyor PDA Plus Detector is a full-featured, photodiode array detector. The detector contains a dual-light source: a deuterium lamp for detection in the ultraviolet wavelength range (190 to 360 nm) and a tungsten-halogen lamp for detection in the visible wavelength range (360 to 800 nm).

The settings for the scan data include the following: scan range, step size, scan rate, and bandwidth.

The maximum scan range is from 190 to 800 nm. You can collect absorbance data for every wavelength in the scan range or in wavelength steps as large as 20 nm. You can collect data points for each wavelength in the scan range at scan rates from 0.5 Hz to 20 Hz (1 Hz = 1 data point per second). Decreasing the scan rate for scans significantly reduces the size of a data file. Because scan data is typically acquired for qualitative purposes, a scan rate of 1 Hz is generally adequate.

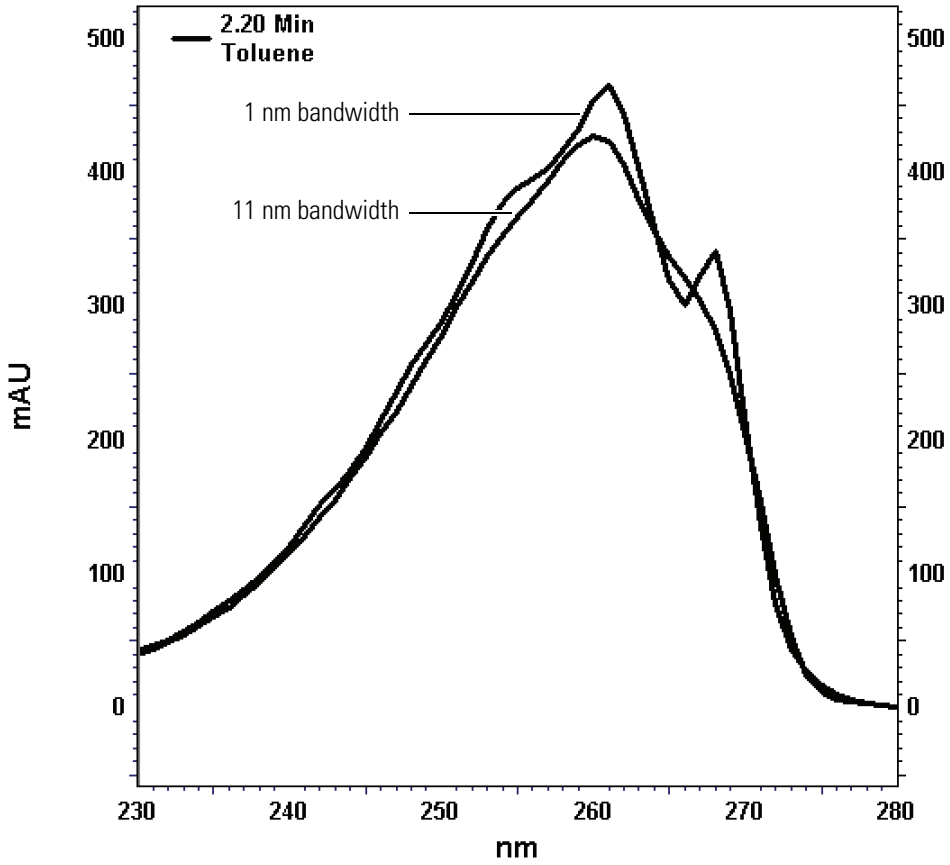
The bandwidth setting is an electronic filter that specifies the range of wavelengths used to determine the absorbance for the central wavelength. Therefore, the maximum allowable bandwidth setting depends on the scan range. For the full scan range of the detector from 190 nm to 800 nm, the maximum allowable bandwidth for each wavelength is 1 nm because the detector cannot monitor wavelengths below 190 nm or above 800 nm. For a scan range from 214 nm to 776 nm, the maximum allowable bandwidth is 49 nm. At a bandwidth setting of 49 nm, the reported absorbance value for 214 nm is a weighted average of the absorbance values from 190 nm to 238 nm. And likewise, the reported absorbance value for 776 nm is a weighted average of the absorbance values from 752 nm to 800 nm.

In addition to scan data, the detector can simultaneously collect up to three independent discrete channels. The data acquisition options for the discrete channels are independent of the scan data, therefore, you can collect the discrete channel data at a higher data rate and a wider bandwidth than appropriate for scan data.

The appropriate data rate setting for the discrete channels depends on the width of the narrowest peak that is integrated. For optimal performance, the integration algorithm in ChromQuest requires twenty points across a peak. If the narrowest peak in your chromatogram is 20 seconds wide, set the data rate for the discrete channels to a minimum of 1 Hz (1 point per second).

Increasing the bandwidth for scans decreases the spectral resolution of the scan data. This is an important factor to consider for compounds that exhibit fine spectral features such as toluene. Figure 1 shows the effect of bandwidth on spectral resolution. Increasing the bandwidth for the discrete channel data decreases the baseline noise. This is an important factor to consider for low concentration samples. For best results, use a lower bandwidth setting for the scan data than for the discrete channels.

**Figure 1.** Effect of bandwidth on resolution




In addition to the bandwidth filter, the detector also contains a computer generated rise-time filter. Increasing the rise time setting can increase the signal to noise ratio for the chromatographic peaks. However, setting the rise time too high can also result in band broadening. In general, a rise time setting that is greater than one-tenth the width of the chromatographic peak at half-height will cause band broadening. For most HPLC applications, a rise time setting of 1 or 2 seconds is preferable.

Figure 2 shows the effect of rise time on peak broadening. The effect of rise time on baseline noise is shown in Table 1.

**Figure 2.** Effect of Rise Time on peak broadening

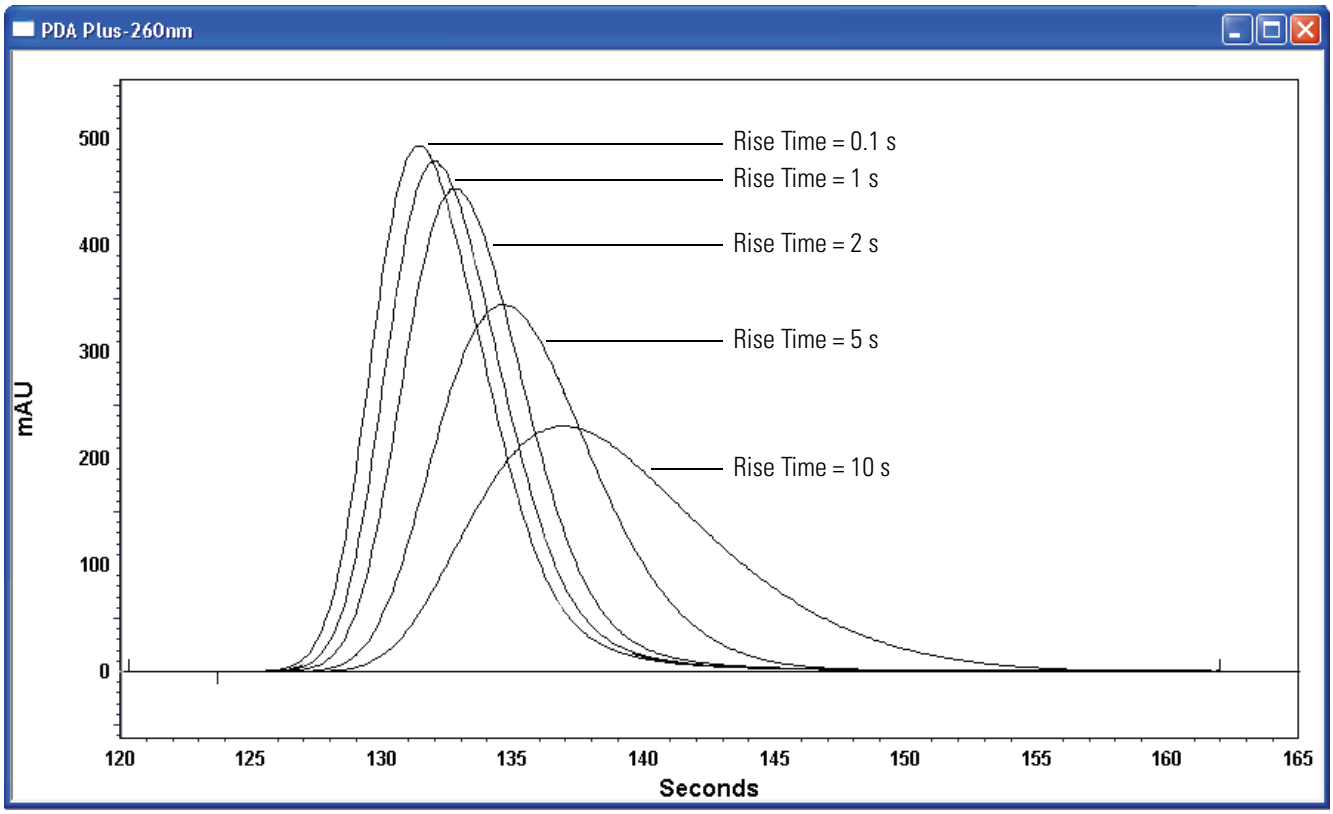



**Table 1.** Effect of rise time on the signal to noise ratio

| Rise Time (s) | Signal to Noise Ratio (ASTM) |
|---|---|
| 0.1 | 7543 |
| 1.0 | 18004 |
| 5.0 | 22654 |
| 10.0 | 72350 |

# Surveyor UV/Vis Plus Detector

The Surveyor UV/Vis Plus Detector is a full-featured, time-programmable, variable-wavelength UV/Vis (ultraviolet / visible) absorbance detector. It operates in either the single wavelength mode or the dual wavelength mode. The wavelength range in the single wavelength mode is 190 nm to 800 nm. In the dual wavelength UV mode, the range is 190 nm to 365 nm. In the dual wavelength Visible mode, the range is 366 nm to 700 nm.

The wavelength time table is available in all three modes. The time table can contain up to 10 lines. If the Zero On Wavelength Change feature is enabled, the absorbance of the baseline is re-zeroed between each line in the time table, even if the wavelengths remain the same. The absorbance of the baseline is not zeroed between the last two lines in the table.

To provide a complete spectrum of ultraviolet and visible light, the detector uses a deuterium lamp for the UV range (190-365 nm) and a tungsten lamp for the visible range (366-800 nm). The lamps are protected by a cover with a special safety interlock to reduce the possibility of human exposure to harmful UV light.

# Surveyor FL Plus Detector

The Surveyor FL Plus Detector is a full-featured, time-programmable, fluorescence detector that integrates with the Surveyor Plus LC system.

The Surveyor FL Plus Detector consists of a xenon (Xe) lamp light source, dual gratings, a flow cell, and a mercury (Hg) lamp for wavelength calibration. The Hg lamp is protected by a cover with a special safety interlock to reduce the possibility of human exposure to harmful UV light.

**CAUTION** The glass bulb of the xenon lamp contains xenon gas under high pressure. Because it can explode, take care when handling the xenon lamp.

The xenon gas inside the glass bulb of the Xe lamp exerts a pressure of 4 MPa at normal operating temperatures, which means that the glass bulb of the Xe lamp can explode. The xenon lamp is contained within the detector housing, which ensures your safety during normal use of the detector. However, the Xe lamp has a limited operating time and must be replaced occasionally. Before you replace the Xe lamp, allow it to cool to room temperature and put on extra personal protective gear, including a face shield and puncture resistant gloves. See the booklet or the CD stored in the left door of the FL detector or the *Surveyor FL Plus Detector Hardware Manual* for information on installing or replacing the Xe lamp.

**CAUTION** Because the flow cell of the FL detector cannot withstand a backpressure higher than 1MPa (10 bar, 145 psi), do not connect the outlet of the flow cell to another detector.

To collect UV/Vis data in addition to fluorescence data, place the UV/Vis detector on the top of the LC stack, and then connect the outlet line from the UV/Vis detector to the inlet of the FL detector's flow cell.

You can control the fluorescence detector from a keypad on its front panel or from the ChromQuest chromatography data system. A USB communications link connects the detector to the ChromQuest data system. Light emitting electrodes (LEDs) on the front of the module keep you informed of the power, communications, run, and lamp status.

The Surveyor FL Plus Detector has its own specialized maintenance software program that you can access by choosing **Start > All Programs > Chromatography > Maintenance for the FL Plus**. You can use the maintenance software program to monitor the performance of the FL detector and to optimize the emission and excitation wavelengths for your analytes. For information on the maintenance software application, see the *Surveyor FL Plus Detector Hardware Manual*.

# Surveyor RI Plus Detector

The Surveyor RI Plus Detector is a full-featured, low-maintenance, refractive index detector that integrates with the Surveyor Plus LC system. With the addition of this detector to the Surveyor Plus product line, you can detect carbohydrates, alcohols, fatty acids, and other compounds having weak chromophores.

The thermostatically regulated optical bench provides stable baselines, with a drift of $2 \times 10^{-7}$ RIU/ h or less and a noise level of $2.5 \times 10^{-9}$ RIU or less. The tungsten lamp has an operating lifetime of 4.5 years, which means that you can avoid lengthy warm-up periods by leaving the detector on and ready for operation. The operating range of 1.00 to 1.75 RIU allows you to use a broad selection of mobile phases. Table 2 lists the refractive indices of some common mobile phase solvents.

**Table 2.** Refractive Indices of some common mobile phase solvents

| Solvent | RI |
|---|---|
| Acetonitrile | 1.34 |
| Water (20 °C) | 1.33 |
| Methanol | 1.329 |
| Acetic Acid | 1.372 |
| Cyclohexane | 1.427 |
| Hexane | 1.375 |
| Benzene | 1.501 |
| Ethylene glycol | 1.427 |
| Carbon disulfide | 1.626 |
| Tetrahydrofuran | 1.408 |
| Chloroform | 1.443 |

You control and validate the RI detector from the ChromQuest data system. The method editor in ChromQuest provides control of the following detector parameters: the operating temperature from 30 to 50 °C, the rise time from 0.1 to 6 seconds, the data rate from 0.5 to 10.0 Hz, and the signal polarity of the chromatographic peaks. A built-in calibration procedure allows you to validate the accuracy of the detector with a 0.35% by weight sucrose in water solution.

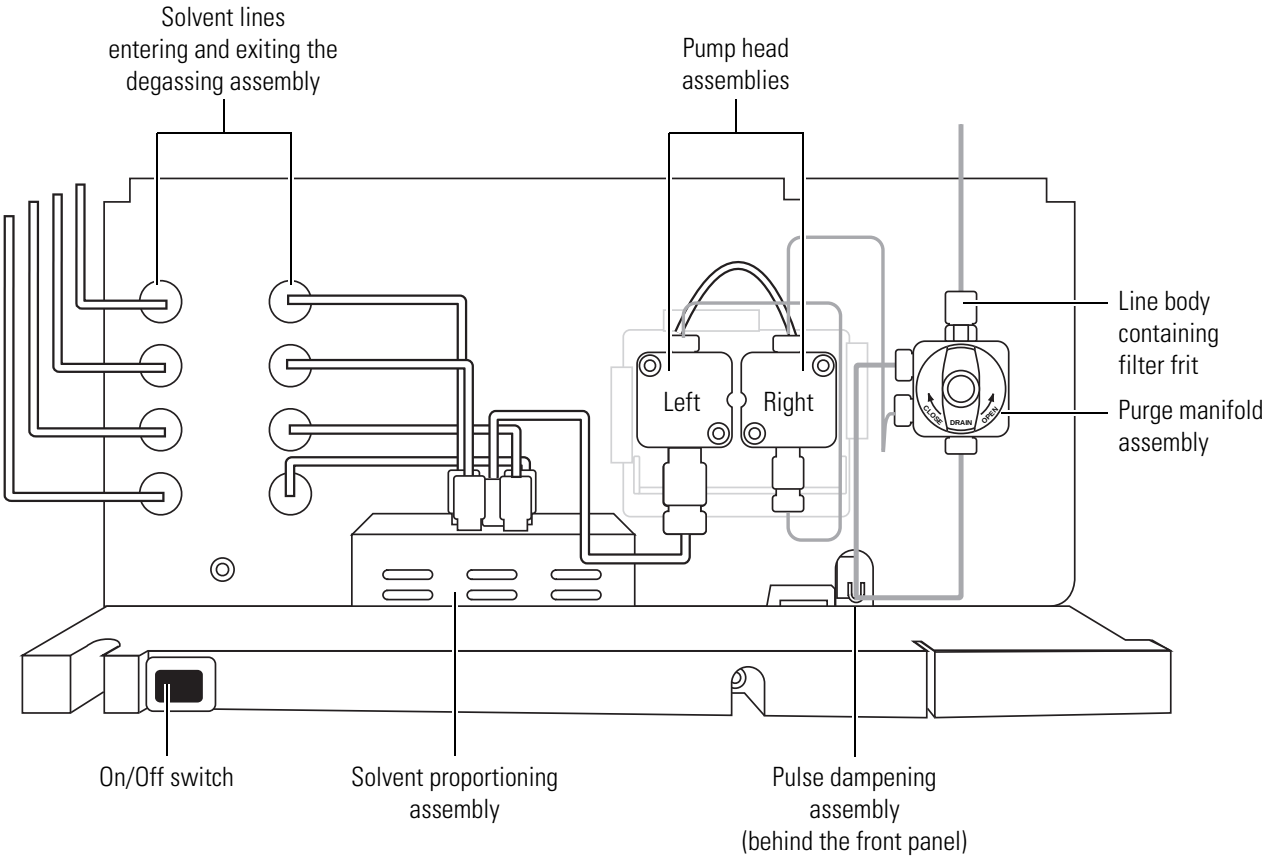
**CAUTION** The flow cell in the Surveyor RI Plus Detector has a pressure rating of 517 kPa (75 psi). Do not connect tubing with an inner diameter of less than 0.060-in. to the OUT port of the RI detector. Doing so can damage the flow cell.

# Surveyor LC Pump Plus

The Surveyor LC Pump Plus is a dual-piston, quaternary, low-pressure mixing pump with a built-in vacuum membrane degasser and pulse dampener. The pumping system provides stable isocratic flow rates from 0.001 to 9.999 mL/min. You can run precise gradients at flow rates from 0.200 to 2.000 mL/min and your gradient table can contain up to forty lines. The integral vacuum degasser offers efficient solvent degassing while requiring only 500 µL of volume, and the pulse dampener produces stable flow rates while adding only 400 µL of gradient delay volume to the system.

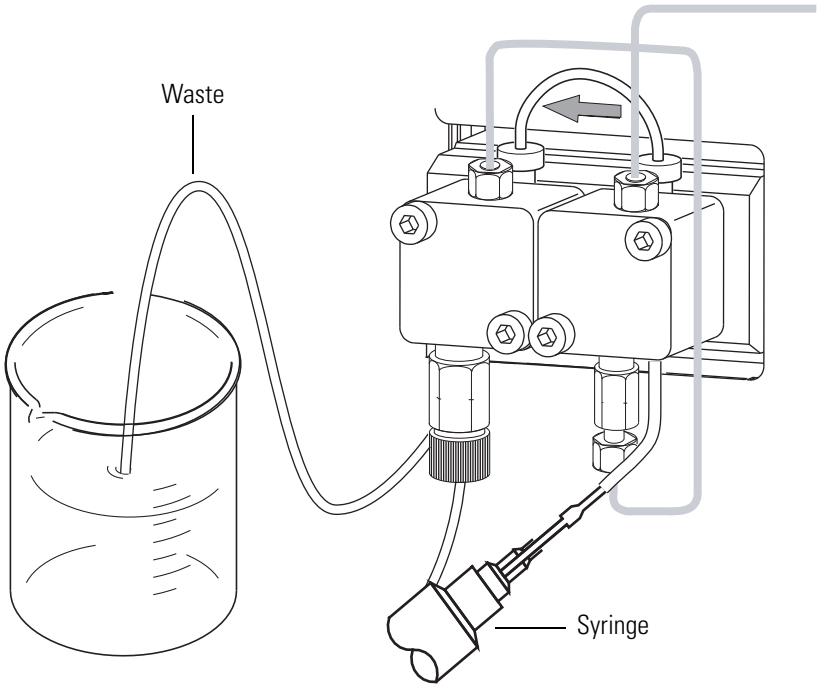
The Surveyor LC Pump Plus, as shown in Figure 3, has five major components: the vacuum membrane degassing assembly, the solvent proportioning assembly, the pump head assemblies, the pulse dampening assembly, and the purge manifold assembly.

**Figure 3.** Front of Surveyor LC Pump Plus



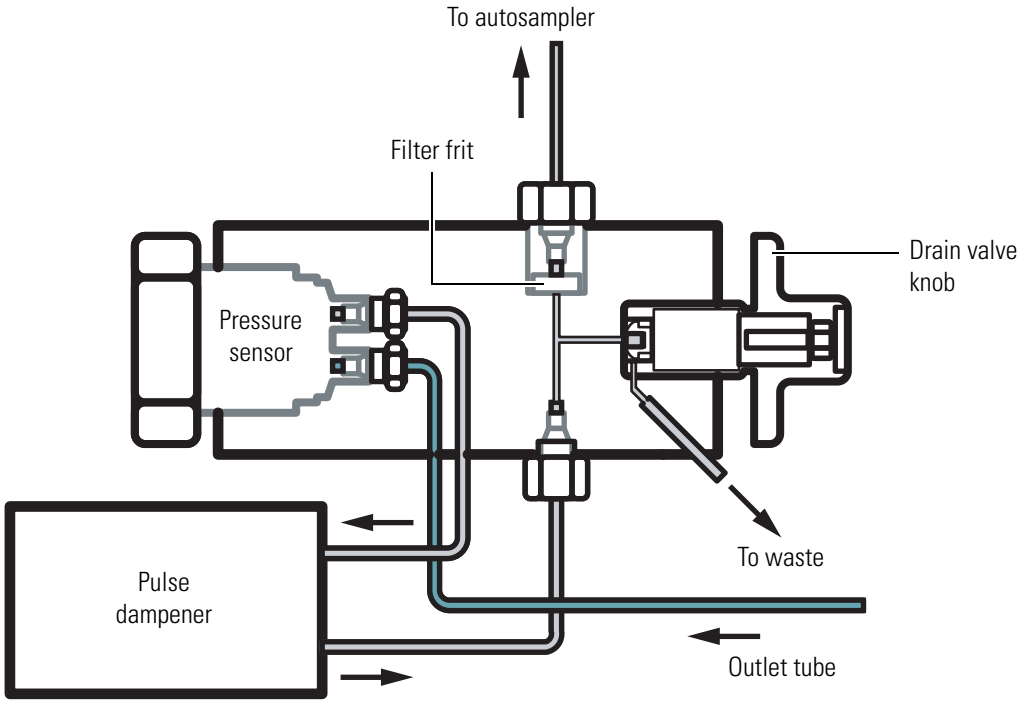

You use the wash tube assembly and the rinse tube assembly to rinse the pump head assemblies with distilled water when you are pumping buffered mobile phases. Rinsing buffered solutions out of the pump heads extends the lifespan of the seals and the pistons. You can rinse the pump heads at any time: while the pump is running or while the pump is stopped. See Figure 4.

**Figure 4.** Set up for rinsing the pump head assemblies




The purge manifold assembly consists of the pressure sensor, the drain valve knob, and the line filter body. The pressure sensor constantly monitors the backpressure of your system. See Figure 5.

**Figure 5.** Purge manifold assembly



The line filter body screws into the top of the purge manifold assembly and holds a replaceable filter frit that prevents particulate matter from entering the injection valve of the autosampler. The particulate matter can come from the mobile phase solvents or from the piston seals as they wear. The backpressure of your system will rise as this filter becomes clogged.

Replace the frit when the system backpressure rises above the typical operating range for your application. How frequently you will need to replace the frit will depend on the purity of your mobile phase solvents and the rate at which the piston seals in your pump wear.

The drain valve knob on the front of the purge manifold assembly can be opened or closed. In the open position, mobile phase flows out the left side of the purge manifold assembly to waste. In the closed position, mobile phase flows out the top of the purge manifold assembly to the autosampler. To open the drain valve, turn the knob 180° counter-clockwise until the word DRAIN is upside down. To close the drain valve, gently turn the knob clockwise until you start to feel resistance. Do not overtighten the knob.

# Surveyor Autosampler Plus

The full-featured Surveyor Autosampler Plus includes a built-in column oven (5 to 95 °C), a tray compartment with temperature control (0 to 60 °C), and automatic sample preparation.

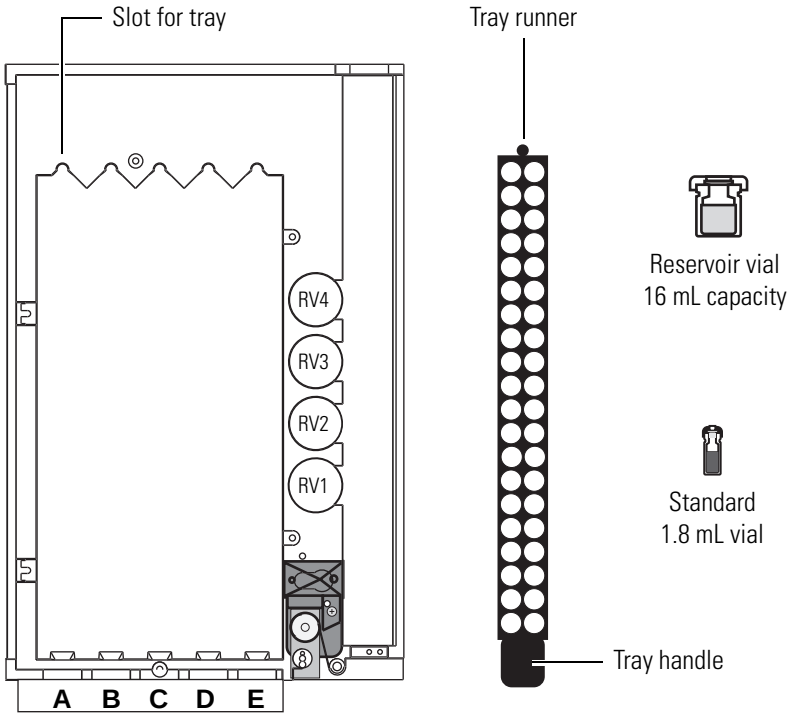
The tray compartment, the injection system, the injection modes, and the temperature control features of the autosampler are described in the following topics:

- Tray Compartment
- Injection System
- Injection Modes
- Temperature Control

## Tray Compartment

The tray compartment can hold up to five conventional sample trays or one microwell carrier. The microwell carrier holds up to three 96-well microplates or up to three 384-well microplates. The tray compartment also holds up to four-16 mL capacity reservoir vials The reservoir vials are located behind the wash station and are designated RV1, RV2, RV3, and RV4. See Figure 6.

**Figure 6.** Tray Compartment




The door to the tray compartment contains a magnetic switch. When you open the door, the switch signals the autosampler that the door is open. If you select the Verify Door is Closed check box when you configure the autosampler, the XYZ arm of the autosampler moves to the back of the tray compartment when you open the to the tray compartment. If you are running a sequence of injections, the sequence pauses until you close the tray compartment door.

# Injection System

The major components of the injection system are as follows:

- Wash Bottle and Wash Bottle Tube
- XYZ Arm Mechanism, Needle Assembly, and Needle Tube Assembly
- Syringe Drive Assembly and Syringe Valve
- Concentric Syringe
- Wash Station
- Needle Port of Autosampler and Transfer Tube
- Injection Valve and Interchangeable Sample Loop

## Wash Bottle and Wash Bottle Tube

The wash bottle is located in the solvent platform mounted on the top of the Surveyor Plus stack. Low pressure tubing connects the wash bottle to the syringe valve. During an injection, the syringe draws solvent from the wash bottle. If the wash bottle is empty, the autosampler cannot withdraw liquid from a sample vial during an injection sequence. To avoid making blank injections, always check the solvent volume in the wash bottle before you begin a sequence of injections.

The following direct commands, **Flush** (from bottle) and **Wash Needle** (from bottle), also draw solvent from the wash bottle.

**Note** If you are using a wash solvent of relatively high viscosity, such as water, reduce the wash speed to 125 µL/s or lower. If you flush a viscous solvent through the injection port at a high speed, the syringe will stall and make a high-pitched screeching sound.

**IMPORTANT** Ensure that there is a sufficient volume of solvent in the wash bottle before performing a sequence of injections.

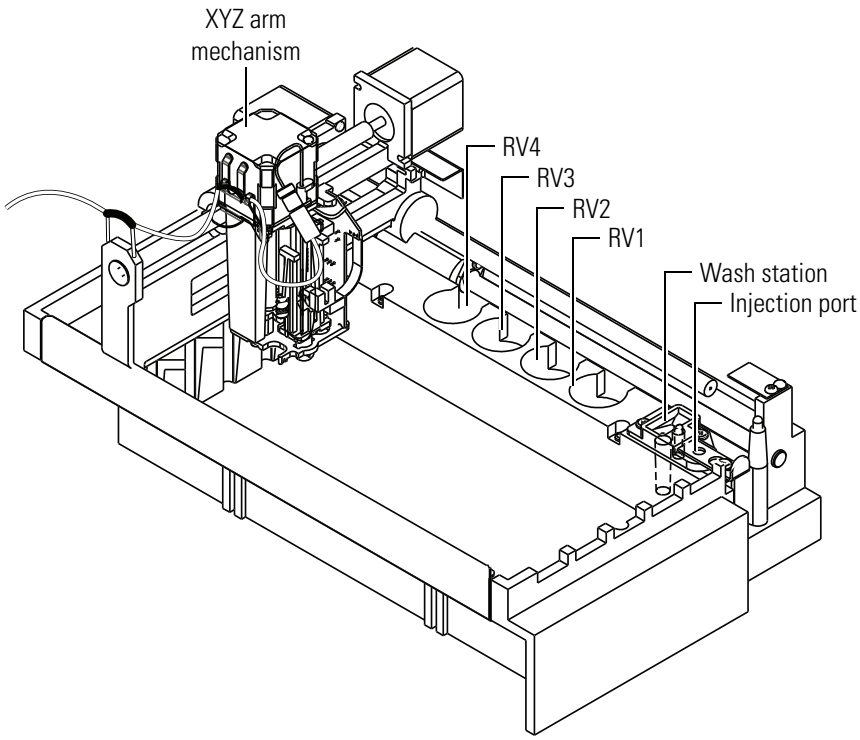
## XYZ Arm Mechanism, Needle Assembly, and Needle Tube Assembly

The XYZ arm mechanism holds the sample needle and moves to the sample location during an injection. The Home position of the XYZ arm is located at the front right-side of the tray compartment, just behind the injection port of autosampler. If you enable the Verify Door Is Closed feature (See "Configuring Your Instrument." ) the XYZ arm moves to the back of the tray compartment when you open the tray compartment door.

When you make an injection, the XYZ arm moves along the X-Y plane to the requested vial or well location, and then lowers the needle to the height requested in the injection method. The inner plunger of the concentric syringe descends, drawing liquid out of the vial, through the needle into the needle tubing. After the autosampler withdraws liquid from the requested well or vial, the XYZ arm returns to the injection port. The autosampler lowers the needle into the injection port, and then the inner plunger of the syringe ascends, expelling the liquid into the injection port.

When you perform a Wash Needle command, the XYZ arm moves along the X-Y plane to the wash station position, located behind the injection port, where it lowers the needle. Confined within the wash station, the needle expels enough wash solvent to clean both its inner and outer surfaces.

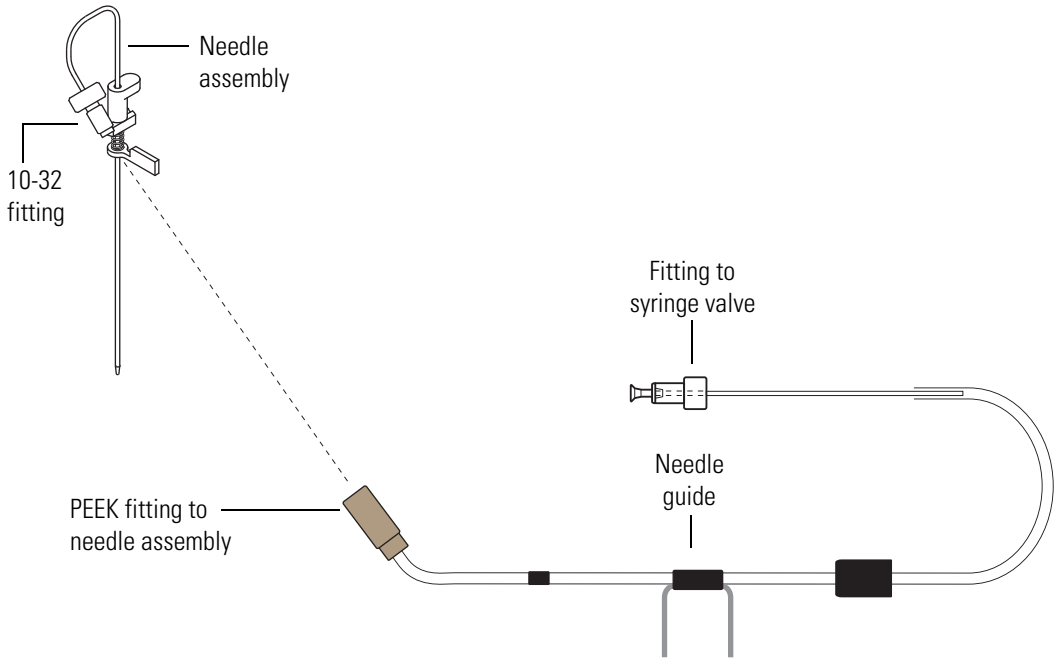
**Figure 7.** XYZ arm mechanism and needle tubing assembly



The needle assembly consists of a blunt-tip needle, a latch nut, a flag, a compression spring, and a 10-32 fitting that connects to the needle tube assembly. See Figure 8. The needle is inserted into the needle mount on the XYZ arm.

The needle tube assembly consists of a piece of low-pressure tubing, a PEEK fitting that connects to the needle assembly, a fitting and flangeless ferrule that connects to the right side of the syringe valve, and a needle tube guide that is inserted into the X-axis positioning frame. See Figure 8. To prevent the needle tubing from interfering with the movement of the XYZ arm, the needle tubing is secured with a bracket on the Z-axis of the XYZ arm and a needle guide on the X-axis positioning frame.

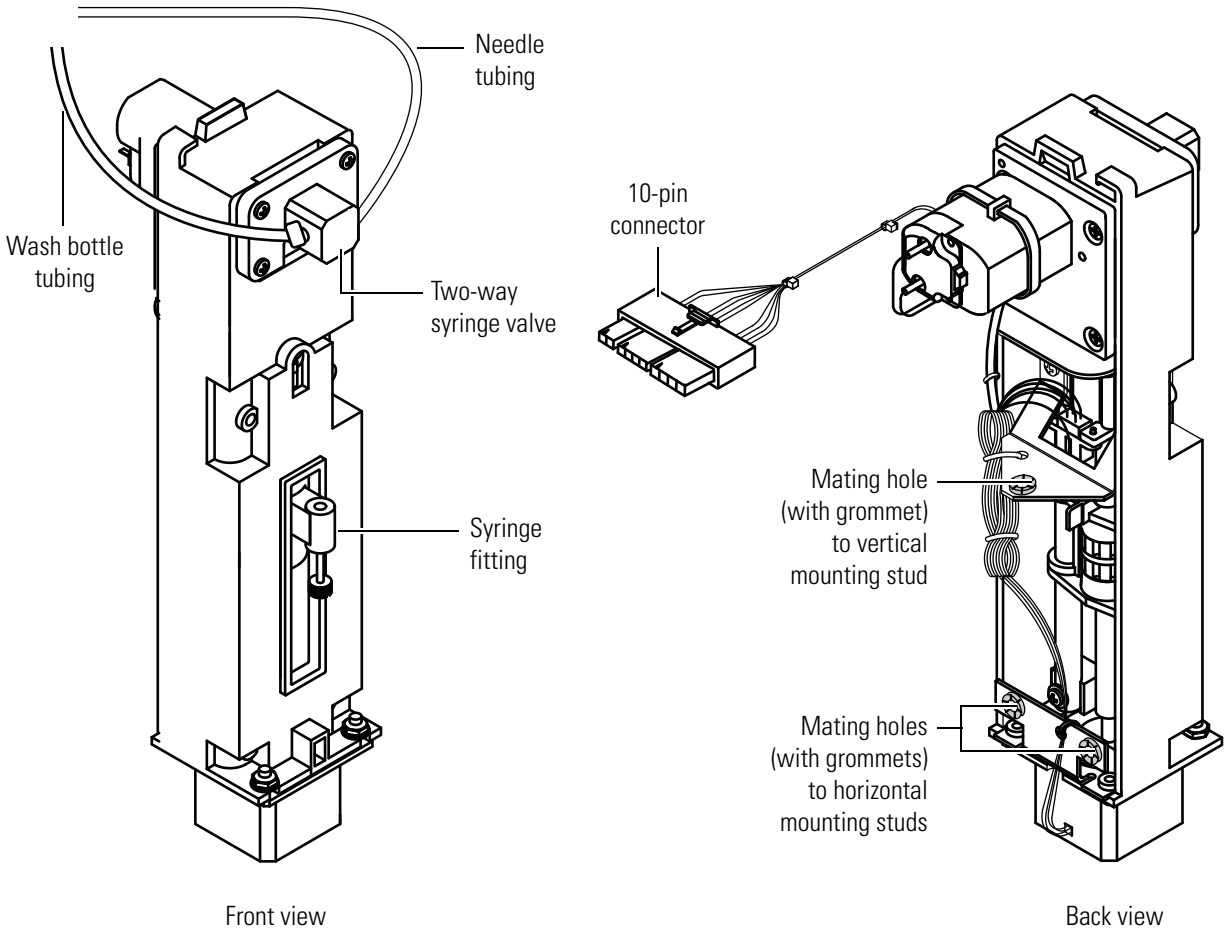
**Figure 8.** Needle assembly and needle tubing assembly



## Syringe Drive Assembly and Syringe Valve

The syringe drive assembly consists of a stepper motor drive mechanism, a syringe valve, and fittings that hold the interchangeable syringe. The syringe drive assembly mounts to the front of the autosampler. The connection between the syringe drive assembly and the body of the autosampler consists of three mating holes with rubber grommets on the back of the syringe drive assembly and three mounting studs on the body of the autosampler. While minimizing vibration, the rubber grommets make the connection between the syringe drive assembly and the autosampler feel loose. See Figure 9.

**Figure 9.** Front and back of syringe drive assembly




The syringe valve is a two-position rotary valve. In the wash bottle position, the syringe draws solvent from the wash bottle into its barrel as its plunger descends. See Figure 10.

In the needle position, the syringe draws sample into the needle tubing as its plunger descends. See Figure 11. Unlike the wash solvent, the syringe does not draw liquid from a sample location into its barrel. As the syringe plunger ascends, it pushes sample solution or solvent out of the needle tubing into the injection port or wash station of the autosampler.

**Figure 10.** Syringe valve in wash tubing position

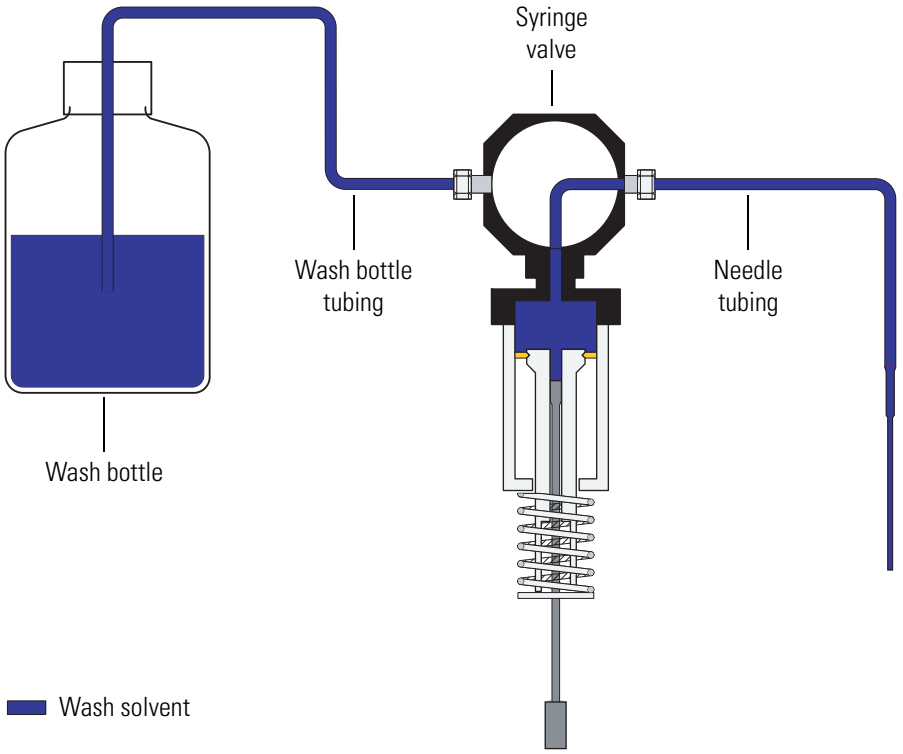
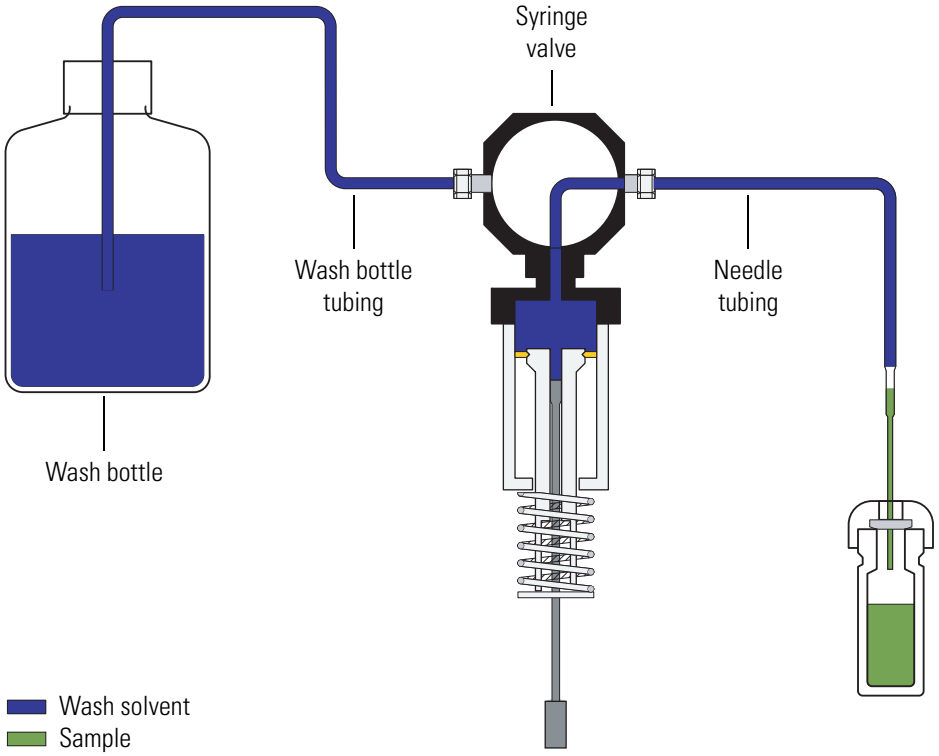



**Figure 11.** Syringe valve in needle tubing position



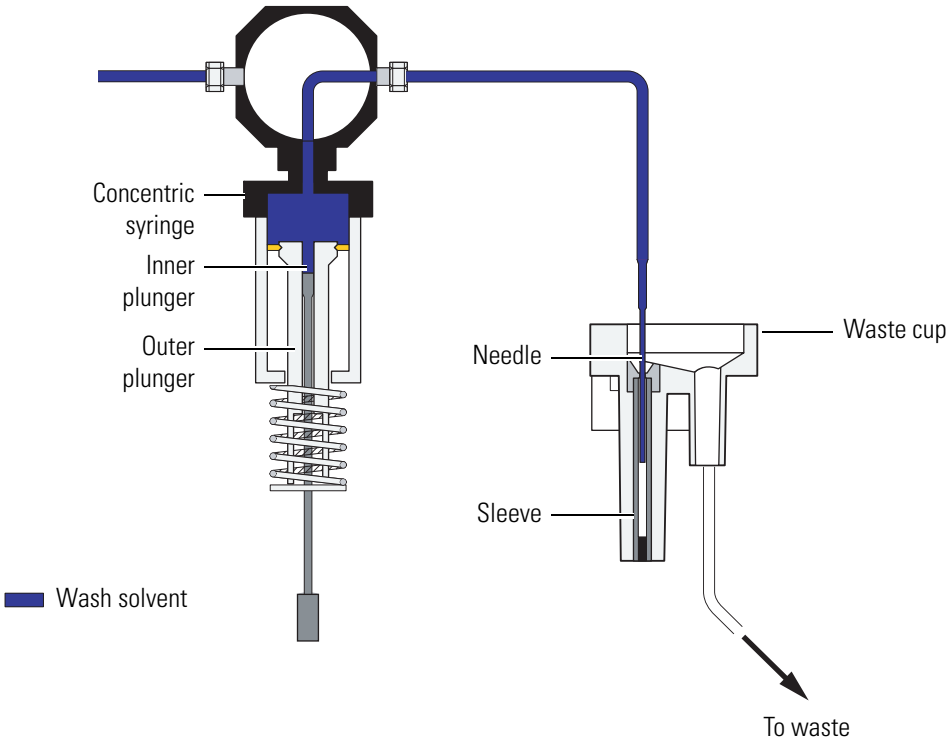
## Concentric Syringe

The Surveyor Autosampler Plus uses a concentric syringe to deliver solvent and sample. See Figure 12. The concentric syringe consists of a small, inner plunger, and a larger, outer plunger. The inner plunger is used to draw sample into the needle tubing assembly, and then deliver the sample to the injection port. The outer plunger is used to draw and expel large volumes of solvent. Liquid from a sample vial is never drawn into the syringe itself.

## Wash Station

The wash station is a waste cup containing an inner sleeve that is slightly larger than the syringe needle. See Figure 12. The waste cup drains into a waste receptacle. The XYZ arm moves the needle to the wash station to perform an external needle wash or to initialize the syringe. The needle is inserted down into the sleeve and solvent is flushed through the needle. This process washes the needle exterior as expelled solvent flows up past the exterior surface of the needle and into the waste cup.

**Figure 12.** Wash station



## Needle Port of Autosampler and Transfer Tube

The injection port of the autosampler is located behind the syringe drive assembly. The injection port is connected to the six-port Valco injection valve by way of the transfer tube. The Surveyor Autosampler Plus has a a 0.012-in. ID transfer tubing. The volume of the transfer tube assembly is specified on its attached label. See Figure 13 and Figure 14.

**IMPORTANT** The volume of each transfer tube is individually calibrated. Enter the volume specified on the label attached to the transfer tube assembly when you configure the autosampler. See "Configuring the PDA Detector" on page 71.

**Figure 13.** Needle port and transfer tube

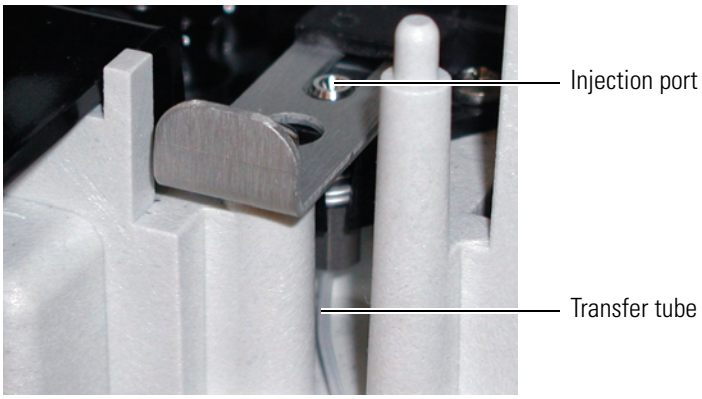
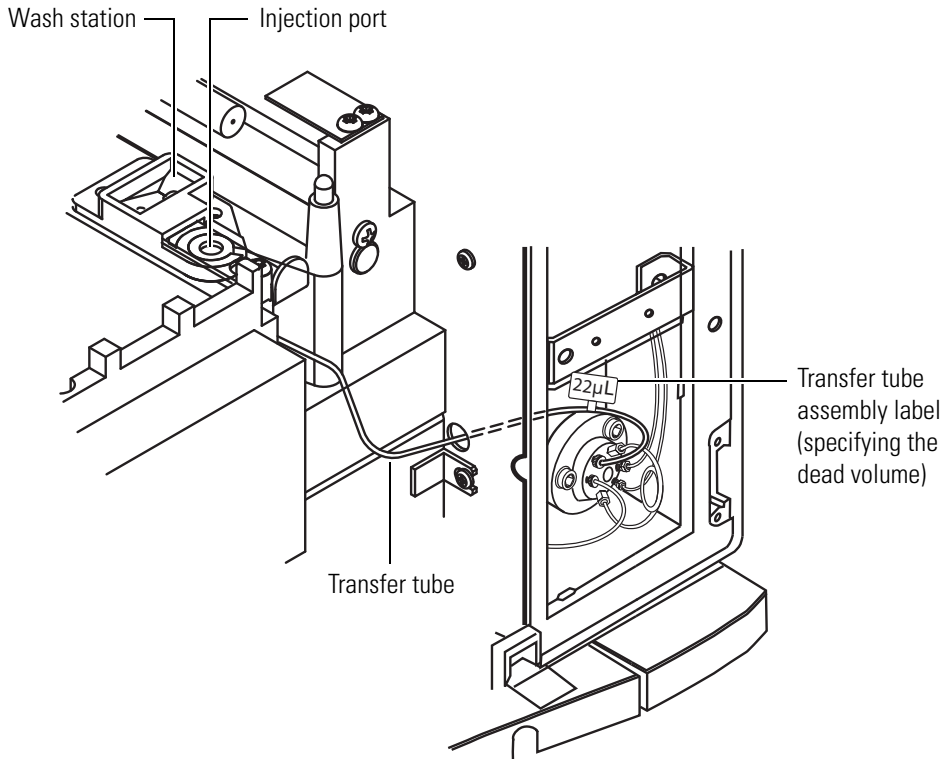



**Figure 14.** Injection port and transfer tube

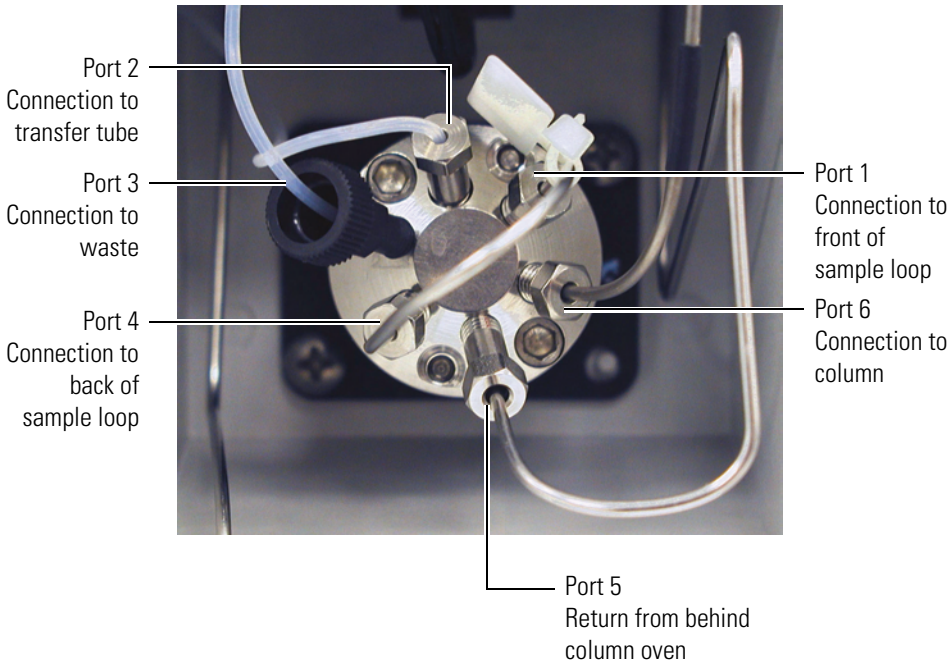


### Injection Valve and Interchangeable Sample Loop

The injection valve is a six-port rotary valve that introduces sample onto the column by way of the sample loop. See Figure 15.

**Note** Because the rotor portion of the valve contains narrow passages, it is important that you remove particulate matter from your samples before you load them into the autosampler.

**Figure 15.** Valco C2 type six-port injection valve




The sample loop is an interchangeable part that is attached to the six-port injection valve. It is a stainless steel tube that holds the sample prior to its introduction onto the column. The Surveyor Autosampler Plus has a 25 µL sample loop.

## Injection Modes

The Surveyor Autosampler Plus can operate in any of the following three injection modes:

- No Waste Injection Mode
- Partial Loop Injection Mode
- Full Loop Injection Mode

The optimum injection mode depends upon the amount of sample that you have and the degree of precision that your application requires.

## No Waste Injection Mode

The no waste injection mode is a technique that withdraws only the exact amount of sample requested from the sample vial. Of the three injection modes, the no waste injection mode uses the least amount of sample, but it is also the least precise. Use this injection mode to conserve sample.

**IMPORTANT** For no waste injections, do the following:

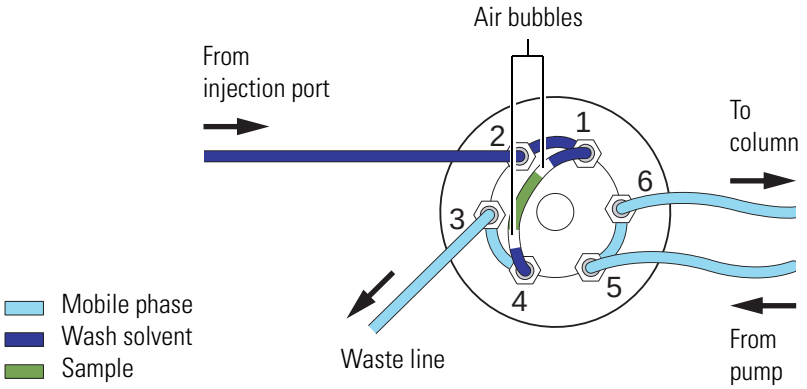
- Use a sample loop that is at least 5 µL larger than the injection volume. Because the accuracy of the nominal size is ± 20%, use an estimate of 80% for the actual size. For example, use 20 µL as an estimate for the actual volume of a 25 µL loop, and inject no more than 15 µL with this loop size.
- Consider matching the chemistry of the sample matrix, the flush solution, and the mobile phase. For no waste injections, the autosampler loads approximately 2 µL of flush solvent and 3 µL of air into the sample loop, regardless of the requested injection volume.
- Inject at least 1.0 µL of sample.

Approximately 0.25 µL of the sample is lost as it travels from the injection port through the transfer tubing and into the injection valve. Because of this loss, inject at least 1.0 µL of sample with the no waste injection mode.

In addition to the sample bolus, the no waste injection mode loads approximately 2 µL of wash solvent and 3 µL of air into the sample loop. These values are independent of the injection volume. Therefore, the chemistry of the wash solvent can affect your chromatographic results. For the best chromatographic results, consider matching the chemistry of the sample matrix, the wash solvent, and the mobile phase. See Figure 16.

**IMPORTANT** For no waste injections, do not inject more than the sample loop size less 5 µL. For example, for a 25 µL loop, do not inject more than 20 µL.

**Figure 16.** No Waste Injection mode, showing the valve in the fill position

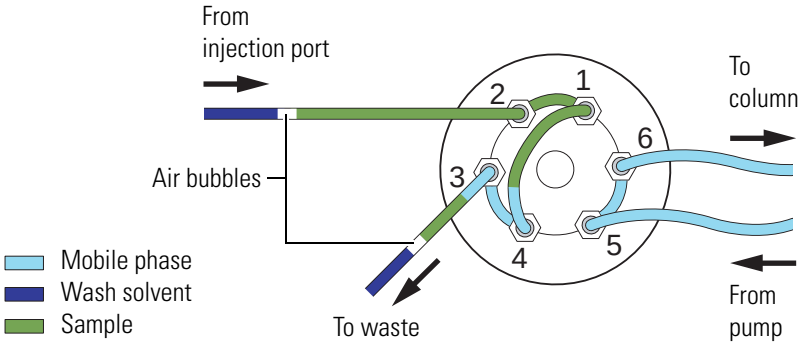


## Partial Loop Injection Mode

The partial loop injection mode is a technique that withdraws 22 µL of excess sample from the vial, in addition to the requested injection volume. Approximately one-half of the excess volume is expelled to waste before the center of the sample bolus is metered into the front of the sample loop. The second portion of excess sample is expelled to waste after the sample bolus is backflushed onto the column. Partial loop injections are useful when you have a limited volume of sample. Using the partial loop injection mode, you can inject variable amounts of sample, ranging from a minimum of 0.1 µL to a practical maximum of one-half the volume of your sample loop. This maximum volume limitation is a consequence of the laminar flow of fluid within the stainless steel sample loop. See Figure 17.

**Note** The actual size of a sample loop can be ±20% of its nominal size. The actual size of the 25 µL sample loop attached to the Surveyor Autosampler Plus is anywhere from 20 to 30 µL. Therefore, limit the maximum injection volume to 10 µL.

**Figure 17.** Partial loop injection mode, showing the valve in the fill position



## Full Loop Injection Mode

The full loop injection mode is a technique that withdraws a sample volume from the vial that is sufficient to overfill the loop by a minimum factor of two. Because the actual injection volume is determined by the size of the loop, not the metering action of the stepper motor, a full loop injection is very reproducible. However, because the intent of the full loop injection mode is to completely fill the sample loop, you cannot inject variable amounts of sample.

Full loop injection is useful when you want maximum precision and have unlimited sample. If you want to change the injection volume, you must change the sample loop size.

**Note** The value you enter in the Loop Size box when you configure your autosampler must match the sample loop size. The injection volume box in the Single Run Acquisition dialog box is disabled for Full loop injections.

In the full loop injection mode, the autosampler withdraws a large excess of solution from the sample vial according to the following equation:

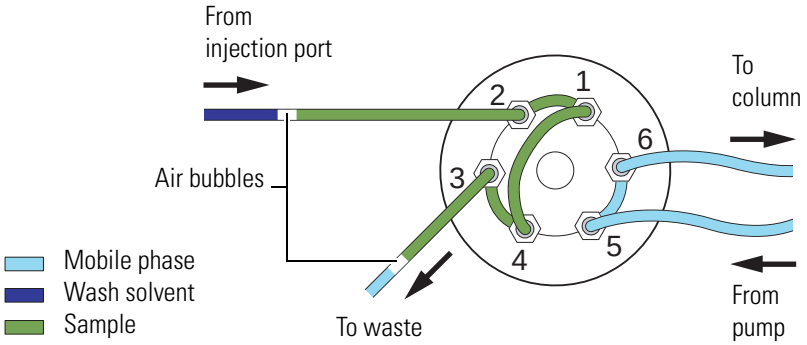
Amount Withdrawn = 3 × Injection Volume + Dead Volume + 7.5 µL

where:

Dead Volume = Volume of Transfer Tube + Volume of Injection Port and Rotor Slot

This equation is valid until the maximum capacity of the syringe is reached, at which point only the maximum capacity of the syringe is withdrawn. The maximum capacity of the 250 µL concentric syringe is 265 µL. See Figure 18.

**Figure 18.** Full loop injection mode, showing the valve in the fill position



## Temperature Control

The full-featured Surveyor Autosampler Plus has two built-in temperature control features:

- Tray Temperature Control
- Column Oven Control

### Tray Temperature Control

The built-in tray temperature control feature provides temperature control of the samples in the range from 0 to 60 °C. A Peltier device maintains the tray temperature.

### Column Oven Control

The built-in column oven controls the temperature of the air surrounding the chromatographic column. Isothermal temperature control is achieved using a Peltier device. The Peltier device is a solid-state, heat-transferring assembly used to heat or cool the column oven. The range of temperature control is 5 to 95 °C.

Between the pump and the autosampler injection valve, the mobile phase is diverted through approximately 122 cm of 0.020-in. ID stainless steel high-pressure tubing that is located behind the column oven. This tubing holds approximately 250 µL of mobile phase, which allows the mobile phase ample time to equilibrate to the temperature of the column oven before it reaches the injection valve. This additional tubing also adds 250 µL of gradient delay volume to the Surveyor Plus System.

# Status LEDs

Each of the Surveyor Plus LC modules has a panel of four status LEDs (light-emitting diodes) located on its front-right door.

All the modules have a Power, Comm, and Run LED. In addition, the PDA, UV/Vis, and FL detectors have a Lamp(s) LED; the autosampler and RI detector have a Temp LED; and the pump has a Degas LED.

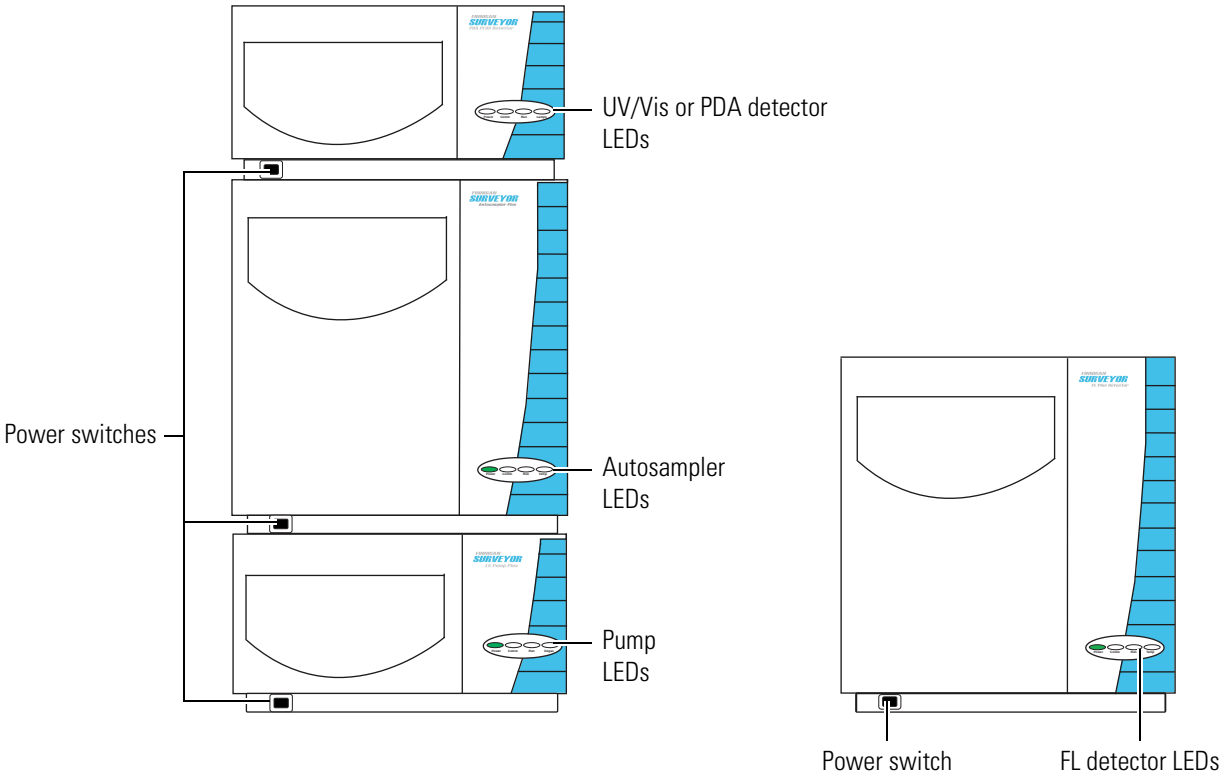
The states of the LEDs are described in the following topics:

- Power LED
- Comm LED
- Run LED
- Lamp(s) LED
- Temp LED
- Degas LED

## Power LED

The Power LED informs you whether the module is turned on or off. The Power LED is unlit before you turn on the module's power switch and remains a steady green after you turn on the power switch. See Figure 19.

**Figure 19.** Location of power switches and LEDs



## Comm LED

The Comm LED informs you whether the module is communicating with the data system. The Comm LED has the states described in Table 3.

**Table 3.** Comm LED states

| State | Module | Description |
|---|---|---|
| Steady green | All modules | The module is communicating with the data system. When you open the online Instrument window in ChromQuest, the Comm LEDs for the configured modules of the instrument should turn green. |
| Steady amber | All modules | The module is not communicating with the data system. To communicate with the data system, the communication cable for the module must be connected and the online Instrument window in ChromQuest must be open. |
| Flashing amber | UV/Vis and PDA detector | The rotary switches on the back panel of the detector are set to 0 for a firmware download. |

## Run LED

The Run LED has the states described in Table 4.

**Table 4.** Run LED states (Sheet 1 of 2)

| State | Module | Description |
|---|---|---|
| Steady green | All modules | The power to the module is turned on. |
| | Pump | A run is not in progress. |
| | Detector | The detector is not sending data to the data system PC. |
| Flashing green | Detectors | The detector is sending data to the data system PC. |
| | Autosampler | An injection or a timed event is in progress. |
| | Pump | A run is in progress. |

**Table 4.** Run LED states (Sheet 2 of 2)

| State | Module | Description |
|---|---|---|
| Steady amber | PDA detector | The PDA detector is not ready to start a run. This condition occurs if both lamps are off, the lamp specified in the method fails to turn on, or the wavelength calibration is not valid. |
| | RI detector | The RI detector is not ready to start a run. This condition occurs when the operating temperature has not reached the set temperature. |
| | Pump | The pump is not communicating with the data system, the pump's motor is stopped, the pump's pistons are homing, the pump is stabilizing, or the pump is in the purge mode. |
| Flashing amber | All modules | An error condition has occurred. |

## Lamp(s) LED

The Lamp(s) LED informs you of the status of the detector's lamps. The Lamp(s) LED has the states described in Table 5.

**Table 5.** Lamp(s) LED states

| State | Module | Description |
|---|---|---|
| Steady green | PDA and UV/Vis detector | The deuterium, tungsten, or both lamps are on. |
| | FL detector | The xenon lamp is on. |
| Steady amber | PDA and UV/Vis detector | The lamp specified in the method (deuterium for the UV range or tungsten for the visible range) is not on. |
| | FL detector | The xenon lamp is off. |

## Temp LED

The autosampler and the RI detector have a Temp LED with the states described in Table 6.

**Table 6.** Temp LED states

| State | Module | Description |
|---|---|---|
| Steady green | Autosampler | The Wait for temperature ready check box in the Surveyor AS Configuration dialog box is not selected. See Figure 63.<br><br>Or this check box has been selected and the column oven and tray temperature zones are in equilibrium at the set temperatures. |
| | RI detector | The operating temperature of the detector is in equilibrium at the set temperature.<br><br>Or the detector is under direct control and the On check box in the Temperature control area of the RI Diagnostic page is not selected. See Figure 236. |
| Steady amber | Autosampler | A temperature change is in progress. |
| | RI detector | A temperature change is in progress. |

## Degas LED

The pump has a Degas LED that informs you whether the built-in degassing unit has developed sufficient vacuum to for chromatography to be performed. The states of the Degas LED are as follows:

- Steady green: Sufficient vacuum has developed for chromatography to be performed.
- Steady amber: The degassing unit is building vacuum.
- Flashing amber: The vacuum has dropped below an acceptable limit of the degasser is unable to build vacuum.

# System Interconnect Cable

A system interconnect cable assembly coordinates the run control signals between the Surveyor Plus modules. There are two versions of this cable.The older version of the cable has five combicon connectors labeled as follows: LC PUMP, MS PUMP, A/S, Det, and M/S detector. See Figure 20. The newer version of the cable has seven combicon connectors: two connectors are labeled PUMP; three connectors are labeled DETECTOR; the connector for the autosampler has a A/S tag on its adjacent cable; the unused connector for a mass spectrometer has a M/S tag on its adjacent cable. See Figure 21.

**Figure 20.** System interconnect cable with five combicon connectors

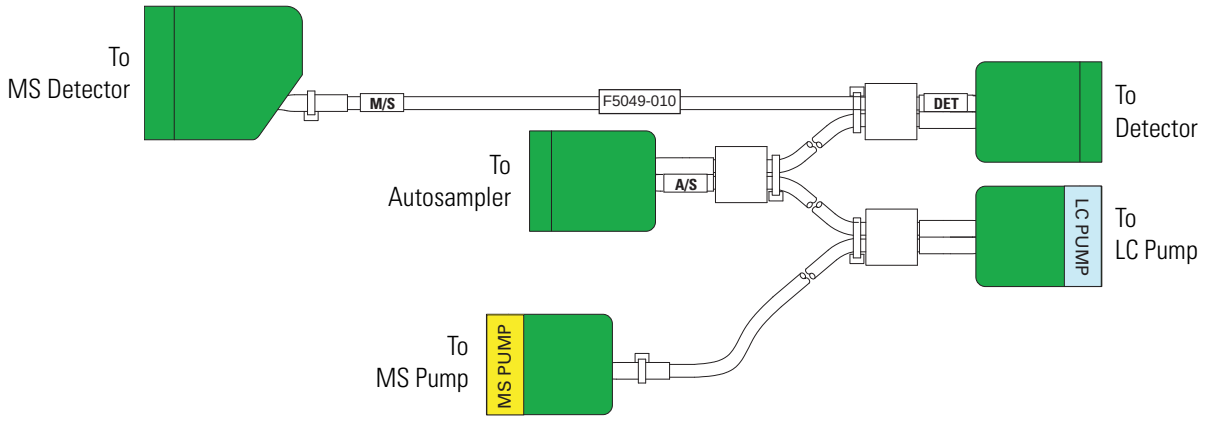
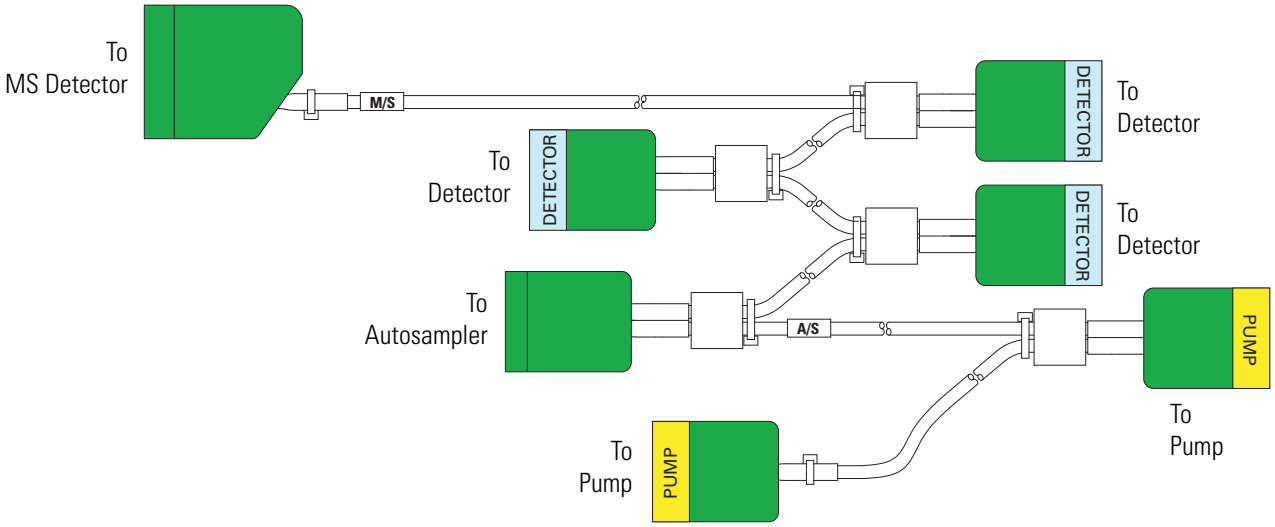


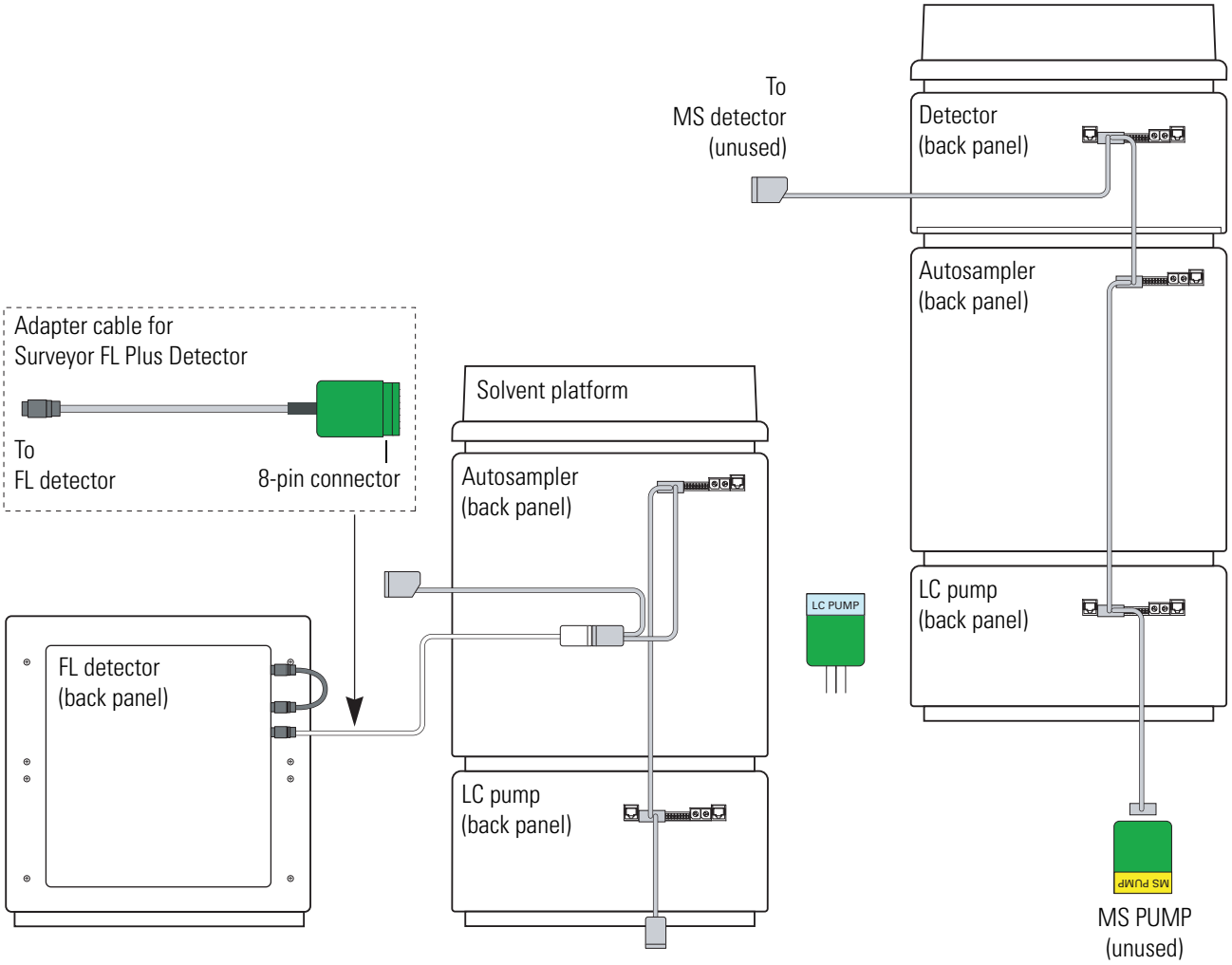

**Figure 21.** System interconnect cable with seven combicon connectors



These connectors attach to an 8-pin connection on the back panel of each module. The MS PUMP and the MS detector connectors are not used for the Surveyor Plus modular HPLC system. To connect the Surveyor FL Plus Detector, you need an additional adapter cable. See Figure 22 and Figure 23.

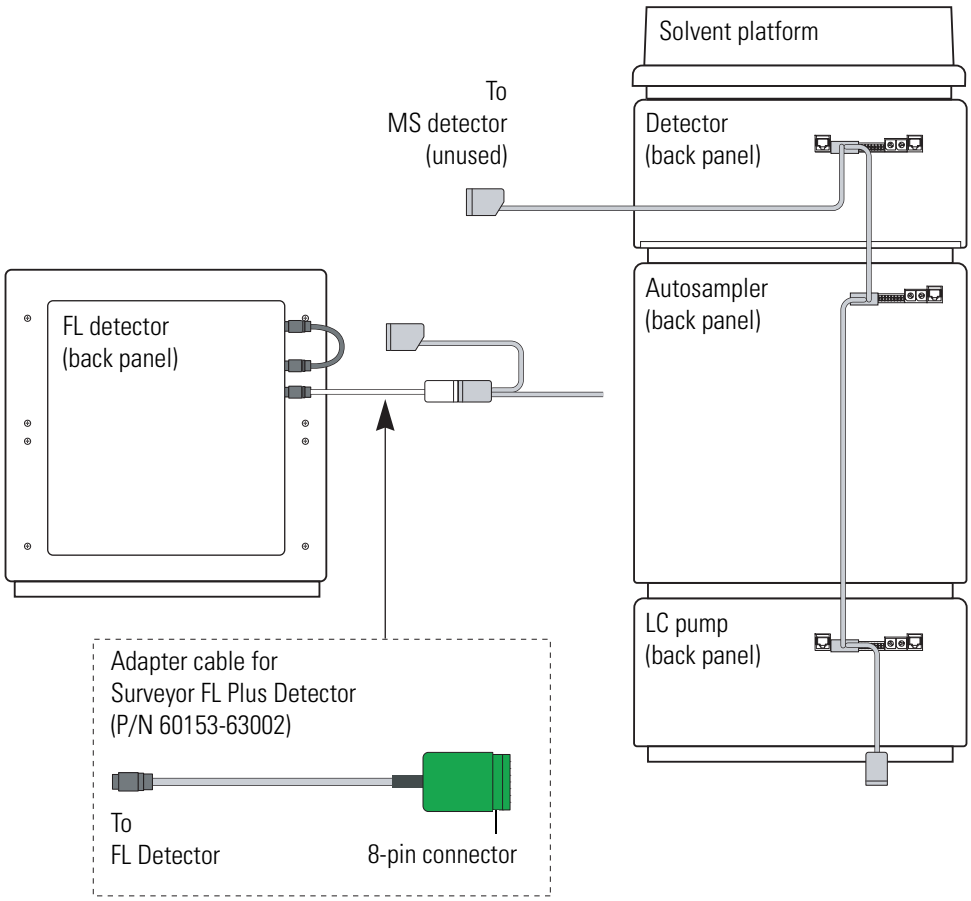
See the *Surveyor Plus Getting Connected Guide* for more information on connecting the interconnect cable.

**Figure 22.** Surveyor Plus modular HPLC system with the five-connector system interconnect cable attached




Not drawn to scale

**Figure 23.** Surveyor Plus modular HPLC system with the seven-connector system interconnect cable attached

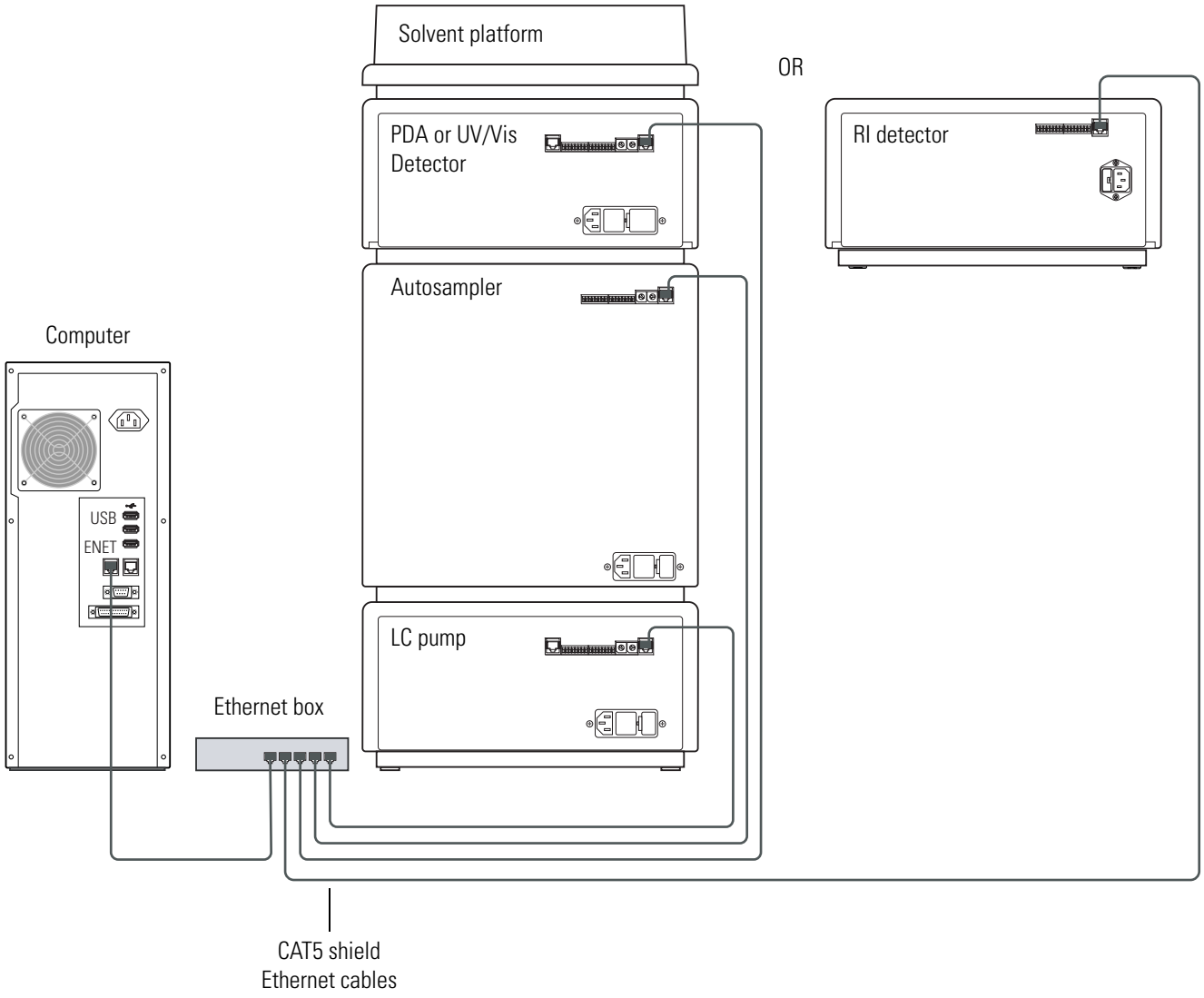



Not drawn to scale

# Communication with ChromQuest

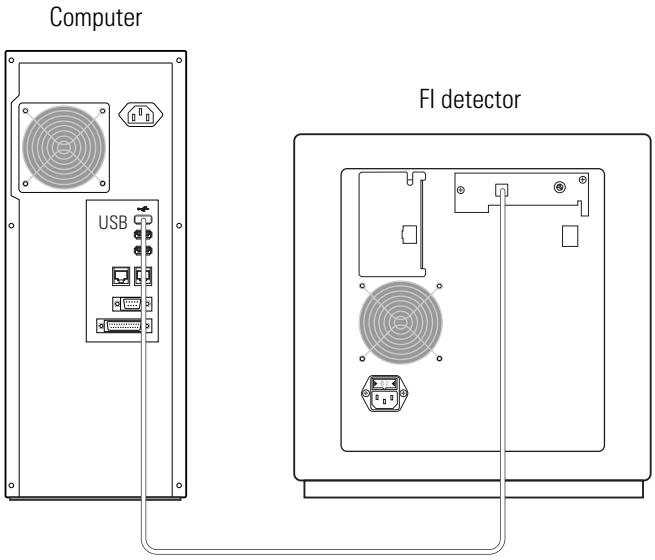
With the exception of the Surveyor FL Plus Detector, the Surveyor Plus modules communicate with the ChromQuest data system by way of an Ethernet connection as shown in Figure 24.

**Figure 24.** Ethernet connections between the Surveyor Plus modules and the data system computer



The Surveyor FL Plus Detector communicates with the ChromQuest data system with a USB connection as shown in Figure 25.

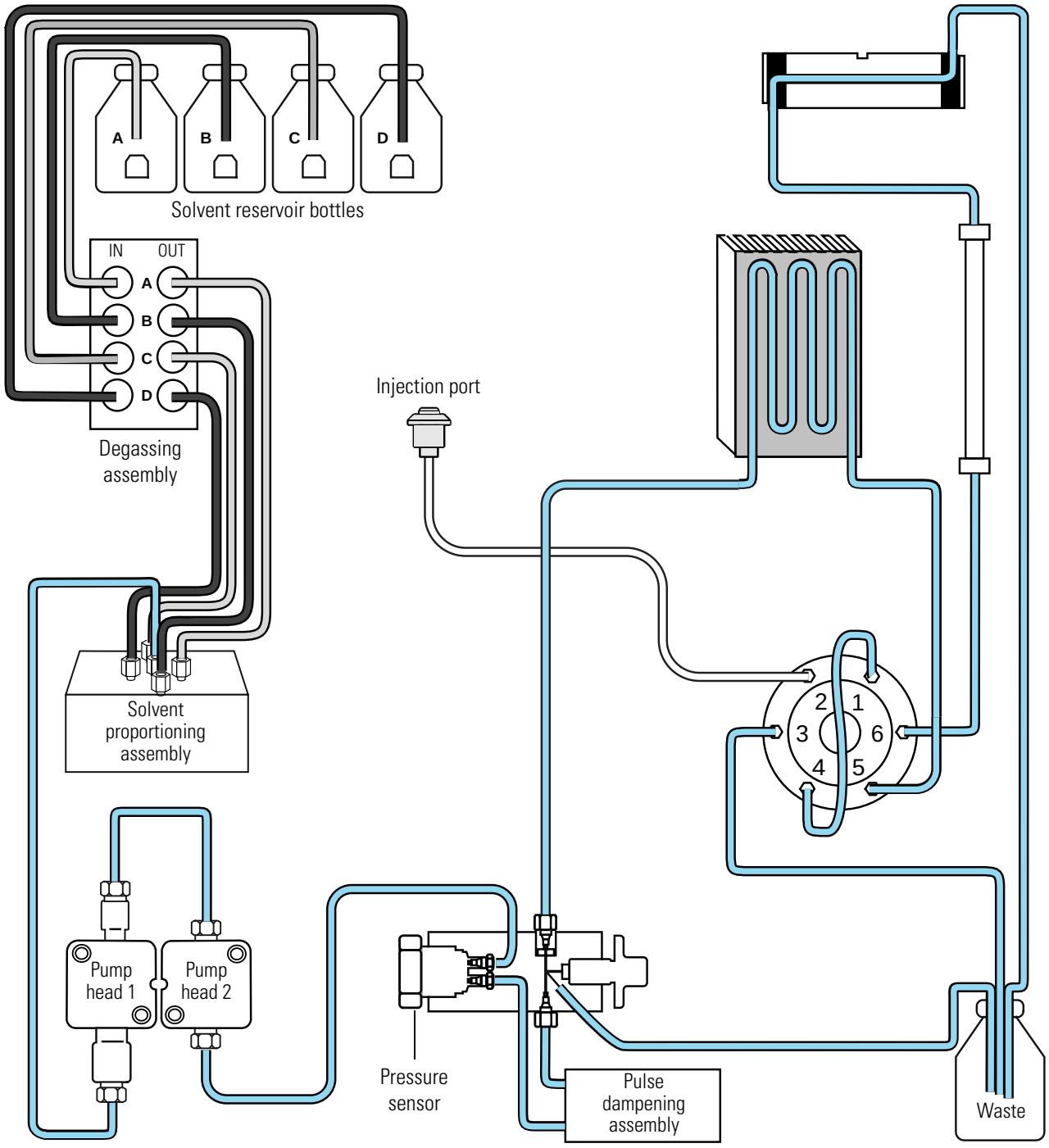
**Figure 25.** USB connection between the FL detector and the data system computer



# Solvent Path

The solvent path of the mobile phase is shown in Figure 26. The mobile phase solvent path begins at the top of the system stack in the solvent reservoir bottles and ends in the waste container as used eluent.

**Figure 26.** Solvent path of the mobile phase



The solvent platform at the top of the Surveyor Plus System stack holds four 1-L solvent reservoir bottles and a 1-L wash bottle. Four low-pressure Teflon solvent lines connect the solvent reservoir bottles to the vacuum degassing assembly. Each of the degassing chambers contains permeable Teflon™ AF tubing with a capacity of 500 µL. Four short low-pressure Teflon solvent lines connect the outlet ports of the degassing chambers to the solvent proportioning assembly.

Each stroke of the pump's pistons draws the solvents that are selected in the method into the solvent proportioning assembly. If you are pumping multiple solvents, mixing is performed in the low-pressure line that connects the solvent proportioning assembly to the inlet check valve. At least one of the four valves in the solvent proportioning assembly is open at all times.

**Note** When you perform routine maintenance tasks, such as replacing the pump seals, you must switch off the power to the pump to close the valves in the solvent proportioning assembly.

As the mobile phase exits the primary pump head, it is under high-pressure. The crossover tube connects the primary pump head to the secondary pump head. The outlet tube connects the secondary pump head to the purge manifold assembly. Both the crossover tube and the outlet tube are constructed of 1/16-in. ID high-pressure stainless steel tubing.

As the mobile phase enters the purge manifold assembly, it passes through a pressure sensor that monitors the backpressure of the system. Then it passes through the pulse dampening assembly, where its pressure pulsations are reduced. As the mobile phase exits the pulse dampening assembly it re-enters the purge manifold assembly.

When the drain valve knob is in the open position, the mobile phase is directed out the left side of the purge manifold assembly to waste. When the drain valve knob is in the closed position, the mobile phase is directed out the top of the purge manifold assembly towards the autosampler. through a stainless steel tube that passes behind the column oven where it loops around the heat exchanger. This tube has a 250 µL volume, which allows the mobile phase to equilibrate to the set temperature of the column oven before it reaches the injection valve.

When the injection valve is in the inject position, mobile phase enters the sample loop from the back, sweeping the liquid in its path out the front of the loop and into the tubing that is connected to the head of the column. When the injection valve is in the fill position, mobile phase bypasses the sample loop.

Red, insulated PEEK tubing connects the end of the column to the inlet port of the flow cell. The insulation on this tubing helps to reduce temperature fluctuations. Blue, PEEK tubing connects the outlet port of the LightPipe flow cell to waste.

**CAUTION** Do not connect tubing with an inner diameter of less than 0.060-in. to the OUT port of the RI detector. Doing so can damage the flow cell. The outlet tubing supplied with the Surveyor RI Plus Detector is 0.060-in. ID × 1/16-in., Teflon tubing.

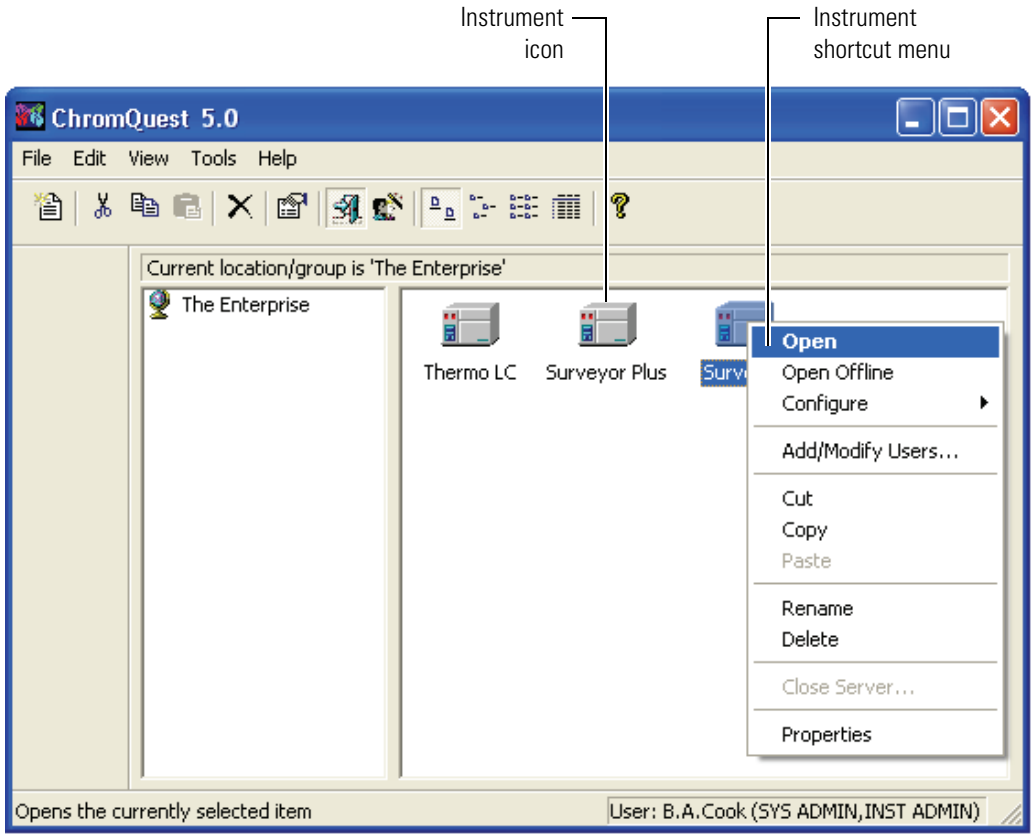
If you are running the pump in the gradient mode, the gradient delay volume of the system begins at the point the mobile phase exits the solvent proportioning assembly. It consists of all the wetted components that lie in the flow path between the solvent proportioning valve of the pump and the inlet of your chromatographic column. This volume is an important factor to consider when developing gradient methods that will be transferred to other instruments. For the Surveyor Plus system, the primary contributors to the gradient delay volume are the pulse dampening assembly of the LC pump (approximately 400 µL) and the tubing that lies behind the built-in column oven of the Surveyor Autosampler Plus (approximately 250 µL).

# Navigation in ChromQuest

The ChromQuest chromatography data system contains two primary windows: the Main Menu window and the Instrument window. You administrate and configure your instrument in the Main Menu window. You control your instrument and process your data in the Instrument window.

Figure 27 shows the Main Menu window, which appears when you launch ChromQuest from the desktop. Right-clicking the icon for your instrument opens a shortcut menu with access to the Configuration dialog box. Double-clicking the icon for your instrument opens the Instrument window, where you perform instrument control and data processing.

**Figure 27.** Main Menu window

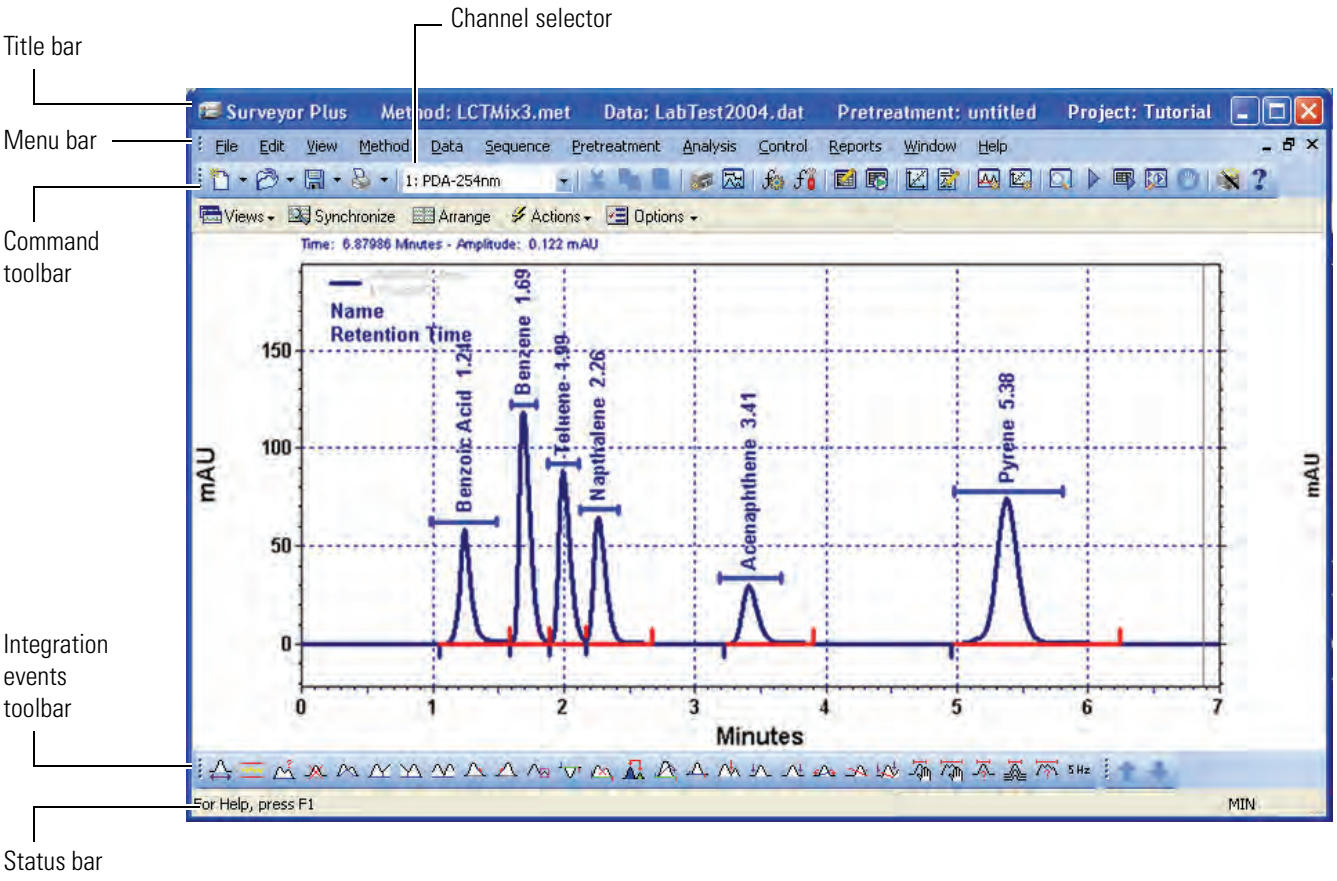


The ChromQuest SI chromatography data system has one main window, the Instrument window. To open the Instrument Configuration dialog box where you specify the software configuration options for the Surveyor Plus LC, choose **Start > All Programs > Chromatography > ChromQuest SI Config**.

The Instrument window shown in Figure 28 contains a chromatogram from a stored data file (LabTest2004.dat) supplied with ChromQuest. The title bar for the Instrument window lists the instrument name, the active method, the active data file, the active pretreatment method, and the project. The Channel Selector box lists the discrete and multi-chromatogram wavelengths contained in the instrument setup section of the method, as well as the scan wavelength that is currently selected, and the spectrum max plot. The command toolbar contains shortcuts to the frequently used features of ChromQuest.

Clicking the Help button, which is located on the far right-side of the command toolbar, opens the online Help for the current window or dialog box.

**Figure 28.** Instrument window, showing the Chromatogram window



# The Tutorials

In addition to the appendix, which contains calibration procedures for the modules of the Surveyor Plus LC system, this manual contains eleven tutorials as follows:

- Chapter 2, "Administrating the Enterprise," shows you how to enable instrument log ins and project management, create users, and create projects.
- Chapter 3, "Configuring Your Instrument," shows you how to add instruments to the Enterprise and configure the modules of your Surveyor Plus system to communicate with the ChromQuest data system.
- Chapter 4, "Creating Methods," shows you how to create acquisition methods for collecting chromatographic and spectral data and shutdown methods for automatically turning off the pump flow and the lamps of the detector.
- Chapter 5, "Preparing Your Instrument for a Run," shows you how to prepare the LC system for a run, which includes removing air from the solvent lines and checking the stability of the chromatographic baseline.
- Chapter 6, "Making Your First Injection," shows you how to load a vial into the sample tray, inject a single sample, and view the results of your injection on the screen.
- Chapter 7, "Adding Integration Events Graphically," shows you how to add integration events to a method and manual integration fixes to a data file.
- Chapter 8, "Specifying the Calibration Curve Parameters," shows you how to add a peak table to the method for the identification and quantitation of your analytes.
- Chapter 9, "Adding a Custom Report to the Method," shows you how to add a custom report to your method so that you can print the results of each injection.
- Chapter 10, "Creating a Sequence Table," shows you how to use the Sequence wizard to create a sequence table for injecting multiple samples. In addition, this chapter shows you how to modify the sequence table by adding a shutdown run or using a different sequence summary report.
- Chapter 11, "Running and Reprocessing a Sequence," shows you how to start and reprocess sequences.
- Appendix A, "Calibration Procedures." shows you how to create a pretreatment method for diluting samples.

If your system administrator has established the security features for ChromQuest on your computer, verify the privileges that have been assigned to you. If you do not have Administrative privileges, skip Chapter 2, "Administrating the Enterprise." If you do not have Instrument privileges, skip Chapter 3, "Configuring Your Instrument."

Security features are not available in ChromQuest SI.

The tutorials contained in chapters 2 through 5 and chapters 7 through 10 can be performed without access to a Surveyor Plus LC system. In addition, the topics "Viewing Your Chromatograms" on page 133, "Viewing Your Spectral Data" on page 135, "Performing a Manual Peak Purity Check" on page 139, and "Reprocessing a Sequence Run" on page 208 can be performed without access to an instrument.

If you do not have a set of data files to work with, use the data files that are supplied with the ChromQuest data system. You can find these tutorial data files in the directory: Drive:\ChromQuest\Data. The data files named multi calibration level 1.dat through multi calibration level 6.dat contain a single chromatogram. The data files named LabTest2001.dat through Labtest2007.dat contain scan data from 220 nm to 360 nm, as well as one discrete channel at 254 nm. For instructions on how to open these data files with their original acquisition method, see "Opening a Stored Data File" on page 146.

In addition to these stored data files, ChromQuest contains the stored sequence table named multilevel calibration.seq. You can find this sequence file in the directory: Drive:\ChromQuest\Sequence.

In chapters 6, 11, and 12, you inject, analyze, and dilute the Autosampler Test Mix (P/N A4991-010), which is a solution of toluene in methanol that is shipped with the Surveyor Autosampler Plus. If you no longer have ampules of the Autosampler Test Mix or you are performing this tutorial to familiarize yourself with the features of the FL or RI detectors, substitute a sample about which you have chromatographic knowledge and adjust the suggested chromatographic parameters accordingly.

# Administrating the Enterprise

In this tutorial, you learn how to enable the security features provided by the ChromQuest 5.0 chromatography data system. In addition, you learn how to create projects to organize your data, methods, sequences, and templates.

ChromQuest SI does not provide security features.

**Note** ChromQuest contains a login security feature that allows the system administrator to assign privileges to users and groups. If this security feature is enabled, users can be denied administrative privileges, instrument privileges, or both. You must have administrative privileges to perform this tutorial. If you are responsible for administrating the data system, make sure that you record your password.

The Enterprise in ChromQuest consists of the instruments controlled from a standalone workstation or the networked instruments administrated from a domain controller. After you define your Enterprise, you must administrate it to enable the security features provided by the ChromQuest chromatography data system.

In addition to the security features that allow you to restrict the access that a user has to the data system, ChromQuest also contains a useful tool called the Project Wizard, which enables you to automatically organize your data, methods, sequences, and templates into project folders.

**Contents**

# Defining the Enterprise

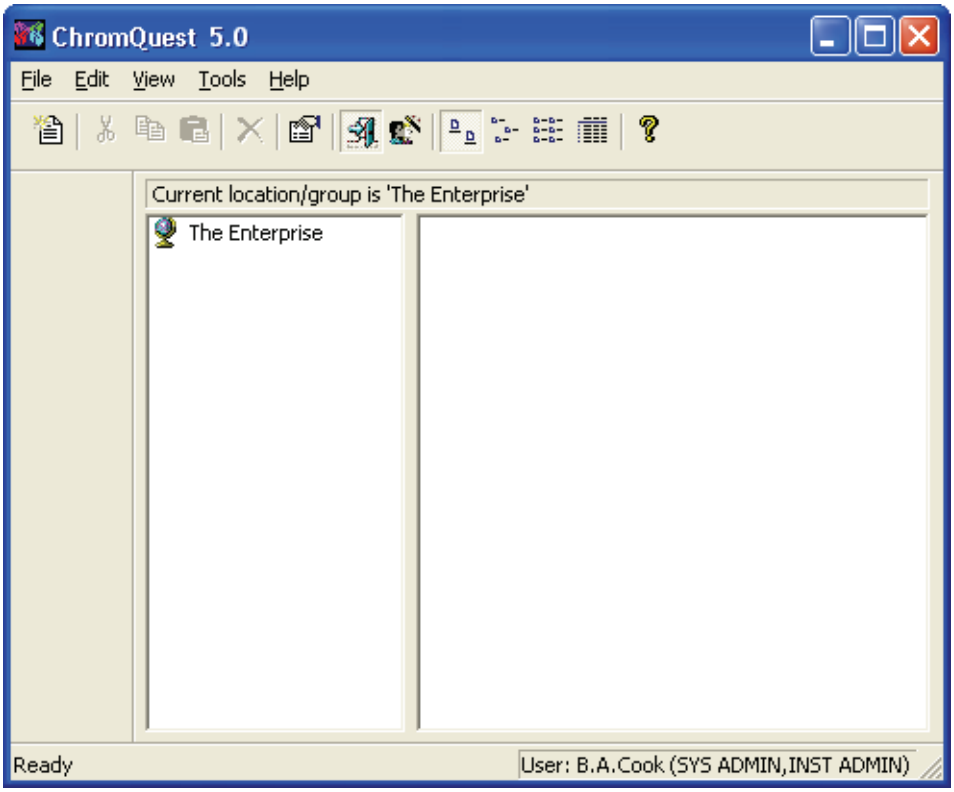
When you install the ChromQuest data system, the Main Menu window has no defined Enterprise and appears, as shown in Figure 29. You or the ChromQuest system administrator must define the Enterprise before instruments can be configured and used. If your Enterprise includes more than one laboratory or group, you might want to add locations to your Enterprise, and then subordinate the instruments by location.

**Note** Because ChromQuest SI controls one instrument, it does not contain the Main Menu window.

Defining the Enterprise involves these two tasks:

- Adding a Location/Group to the Enterprise
- Adding an Instrument to the Enterprise

**Figure 29.** Main Menu window, showing an undefined Enterprise



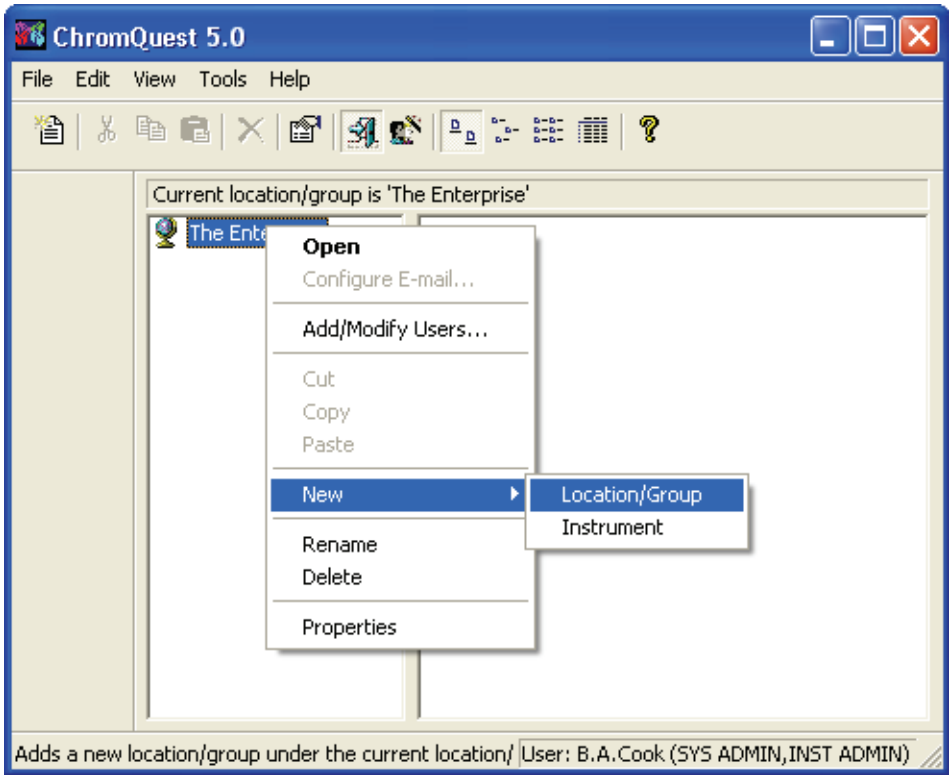
# Adding a Location/Group to the Enterprise

### ❖ To add a Location or Group to your Enterprise

1. Right-click the Enterprise and, choose **New > Location/Group** from the shortcut menu. See Figure 30.

   A New Location subdirectory appears in the left panel of the Main Menu window.

   **Figure 30.** Main Menu window, showing shortcut menu




2. Rename the New Location/Group:
   a. Right-click the new location and choose **Rename** from the shortcut menu.
   b. Type an appropriate name for this Location or Group in the box next to the Location icon, and then press ENTER.

# Adding an Instrument to the Enterprise

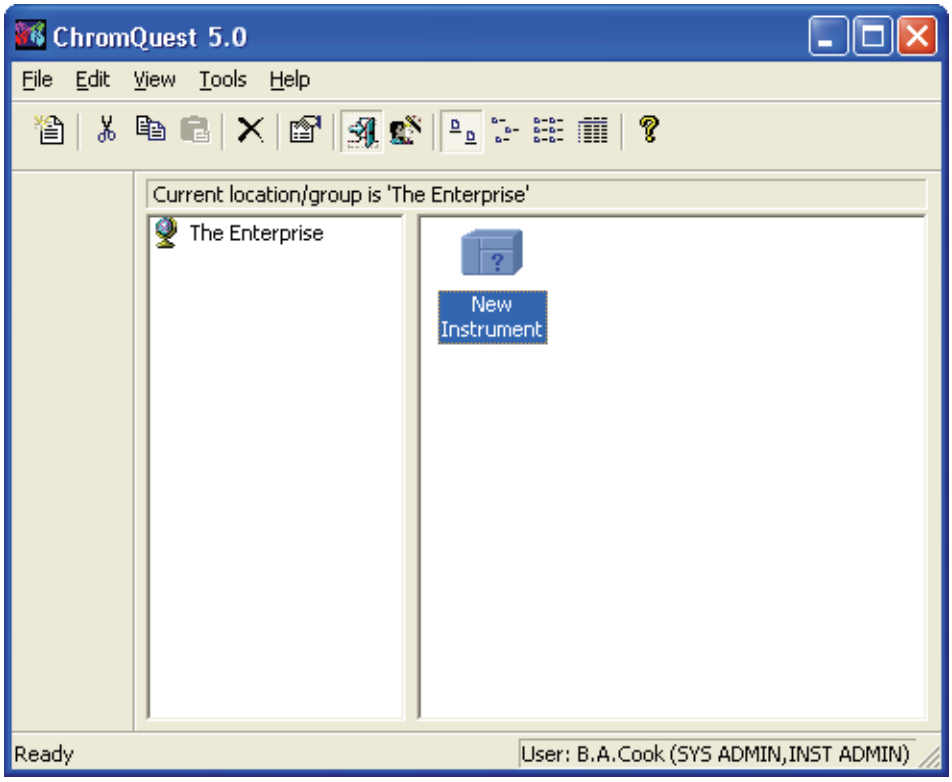
**Note** ChromQuest SI (single instrument) does not support an Enterprise.

❖ **To add an instrument to the Enterprise**

1. Right-click the Enterprise or a Location and choose **New > Instrument** from the shortcut menu.

   A new instrument appears in the right panel, as shown in Figure 31.

   **Figure 31.** Main Menu window with a new instrument




2. Highlight the name in the box below the instrument icon to select it.
3. Type the name **Surveyor System 1** (or your assigned instrument name) in the box, and then press ENTER.

See Chapter 3, "Configuring Your Instrument," for information on configuring your instruments. Instruments that have not been configured are labeled with a question mark.

# Enabling Instrument Login and Project Management

When ChromQuest is initially installed, the security features are not enabled, and everyone has access to all the pages of the Options dialog box and the System Administration Wizard. Although everyone can create projects, the project login feature is not available, which means that projects are not automatically assigned to specific folders.

To automate project management and enable the security features available in ChromQuest, you must enable instrument login and project management.

### ❖ To enable instrument login and project management

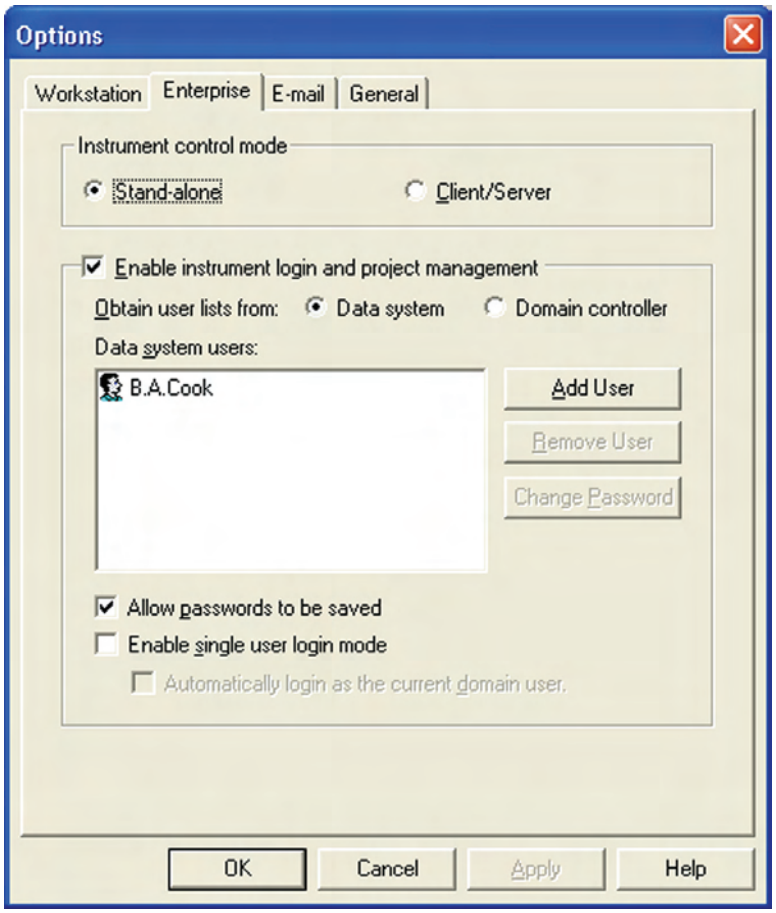
1. From the menu bar in the Main Menu window, choose **Tools > Options**.

   The Options dialog box appears.

2. Click the **Enterprise** tab

   The Enterprise page appears. See Figure 32.

   **Figure 32.** Enterprise page of the Options dialog box




3. Select the **Enable Instrument Login and Project Management** check box.

4. Select the source of the user lists for the Enterprise:

   - If your Enterprise consists of a workstation that is not networked or a workgroup that is not connected to a domain controller, click the **Data System** option, and then continue at the section, "Obtaining User Lists from the Data System Computer" on page 44.
   - If your ChromQuest Enterprise consists of networked workstations connected to a domain controller, click the **Domain Controller** option, and then continue at the section, "Obtaining User Lists from a Domain Controller" on page 46.

**Note** If you do not want to enable the login feature., clear the **Enable Instrument Login and Project Management** check box after you finish this tutorial.

# Obtaining User Lists from the Data System Computer

If your Enterprise consists of a workstation that is not networked or a workgroup that is not connected to a domain controller, you obtain your user list from your data system computer.

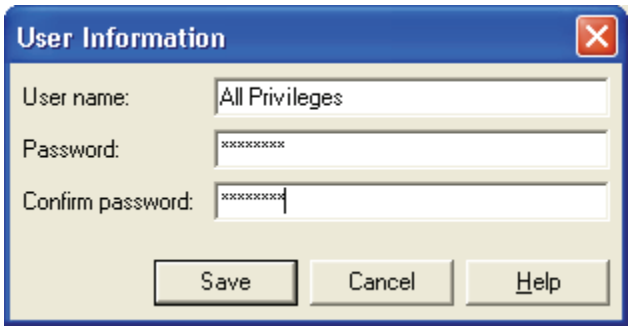
In this tutorial, you create a user list that contains two users.

### ❖ To create the user list

1. In the Enterprise page of the Options dialog box, shown in Figure 32, confirm that the **Data System** option is selected.
2. Click **Add User**.

   The User Information dialog box, shown in Figure 33, appears.

   **Figure 33.** User Information dialog box




3. In the User Information dialog box, do the following:
   a. Type **All Privileges** in the User Name box.

**Note** If you are following these instructions only to familiarize yourself with the administrative features of ChromQuest, leave the Password and Confirm Password boxes blank.

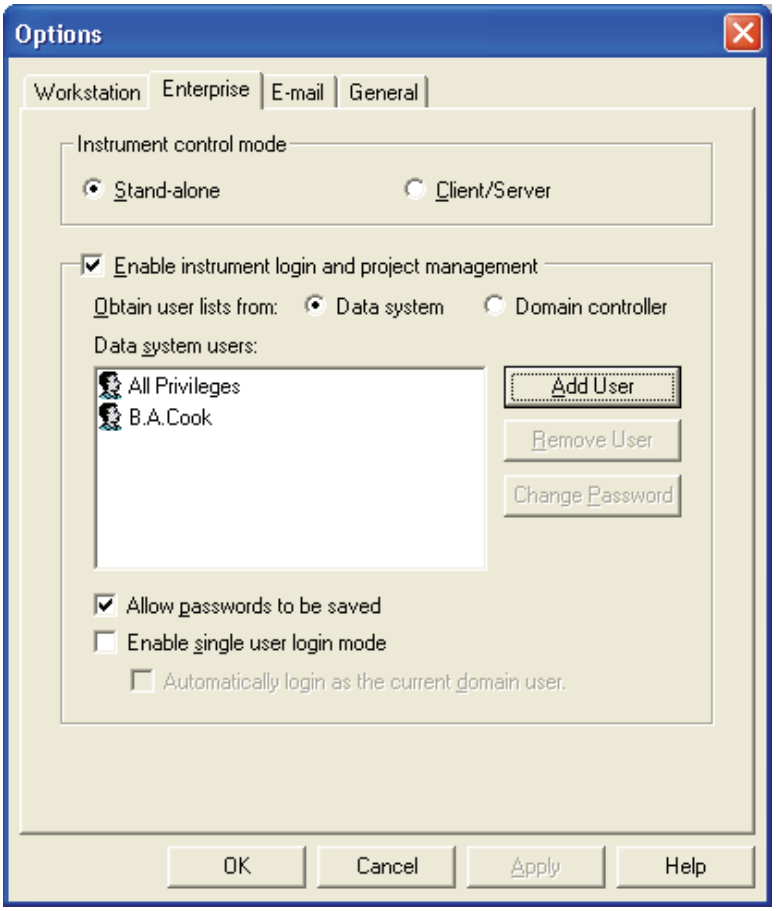
b. Click **Save**.

c. Repeat steps 3a and 3b to add yourself (your name) to the list of Data System Users.

ChromQuest adds the user **All Privileges** and the name you gave yourself to the Data System Users list, as shown in Figure 34.

4. After you finish adding your users to the Data System Users lists, click **OK** to exit the Options dialog box.

**Figure 34.** Enterprise page with a list of Data system users



# Obtaining User Lists from a Domain Controller

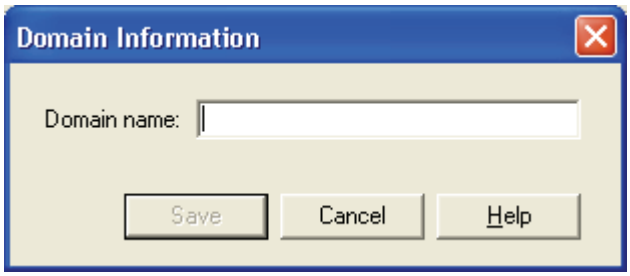
If your ChromQuest Enterprise consists of networked workstations connected to a domain controller, you obtain your user list from the domain controller.

### ❖ To select the domains to be scanned for user information

1. From the Enterprise page of the Options dialog box, confirm that the **Domain Controller** option is selected, and then click **Add Domain.**

   The Domain Information dialog box, shown in Figure 35, appears.

   **Figure 35.** Domain Information dialog box

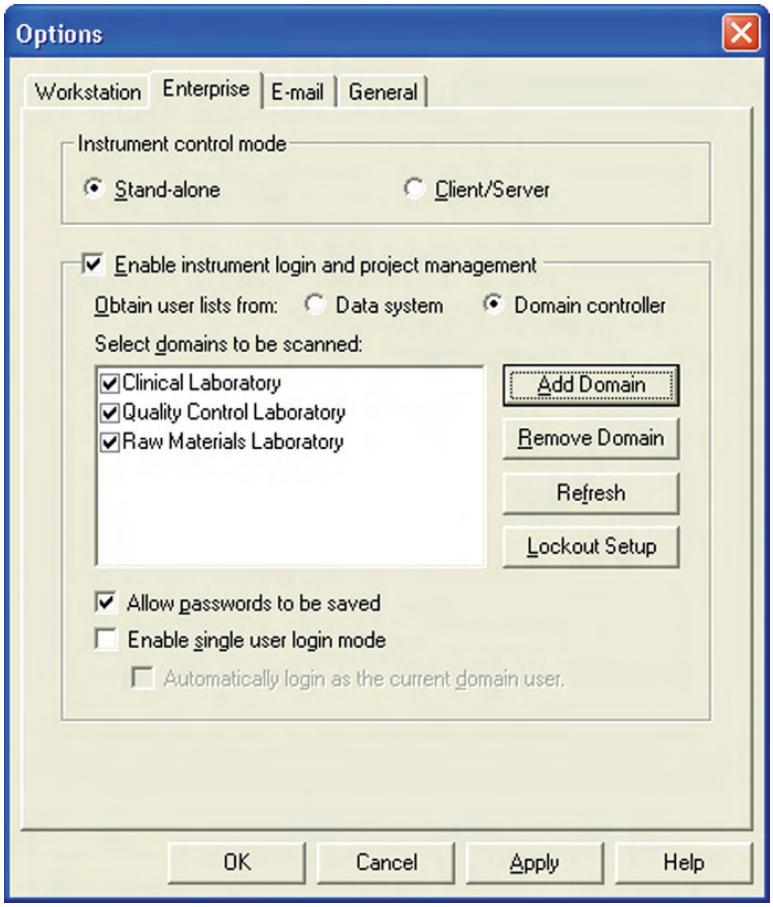
   
   

2. Type the domain name in the Domain name box, and then click **Save**.

   The domain name appears in the list of domains to be scanned, as shown in Figure 36.

3. Select the domains that you want to scan, and then click **OK** to exit the Options dialog box.

**Figure 36.** Enterprise page with a list of domains to be scanned




# Creating a Project

The system administrator has the choice to enable the Login and Project Management feature:

- If the system administrator enables the Login and Project Management feature, you must be a user with system administration privileges to create projects.
- If the system administrator does not enable the Login and Project Administration feature, all users can create and access projects. Users are not required to login and the project management feature is not automated. This lack of automation means users have to confirm that they are storing data files, methods, sequences, templates, and advanced reports in the appropriate directories.

### ❖ To create a project to be used for the tutorials in this manual


Locked


Unlocked

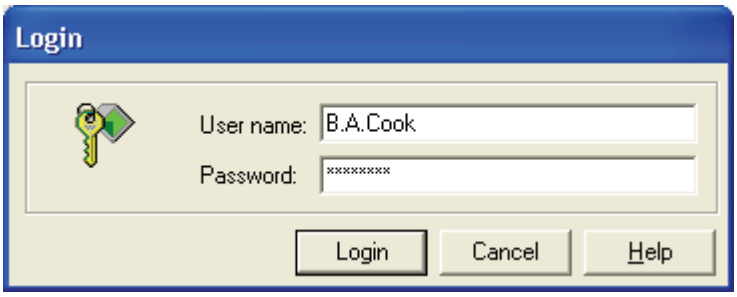
1. If the System Administration Wizard is unlocked, go to step 2. If the System Administration Wizard is locked (grayed out), log in:

   **Note** If your user names are obtained from a domain controller, the Enable Administration login box will contain a list from which you select the domain name.

   a. Click the **Enterprise Login or Logout** button.

      The Login dialog box appears. See Figure 37.

      **Figure 37.** Login dialog box

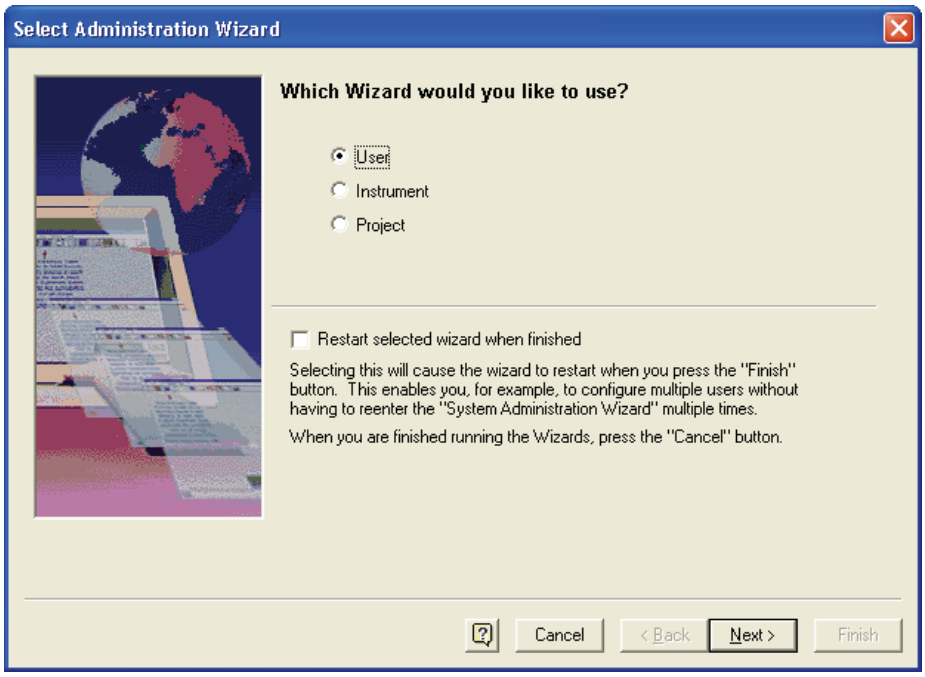
   b. Type your user name in the User Name box and your password in the Password box, and then click **Login**.

      The System Administration Wizard becomes available.

2. Click the **System Administration** button.

   The Select Administration page of the System Administration Wizard appears. See Figure 38.

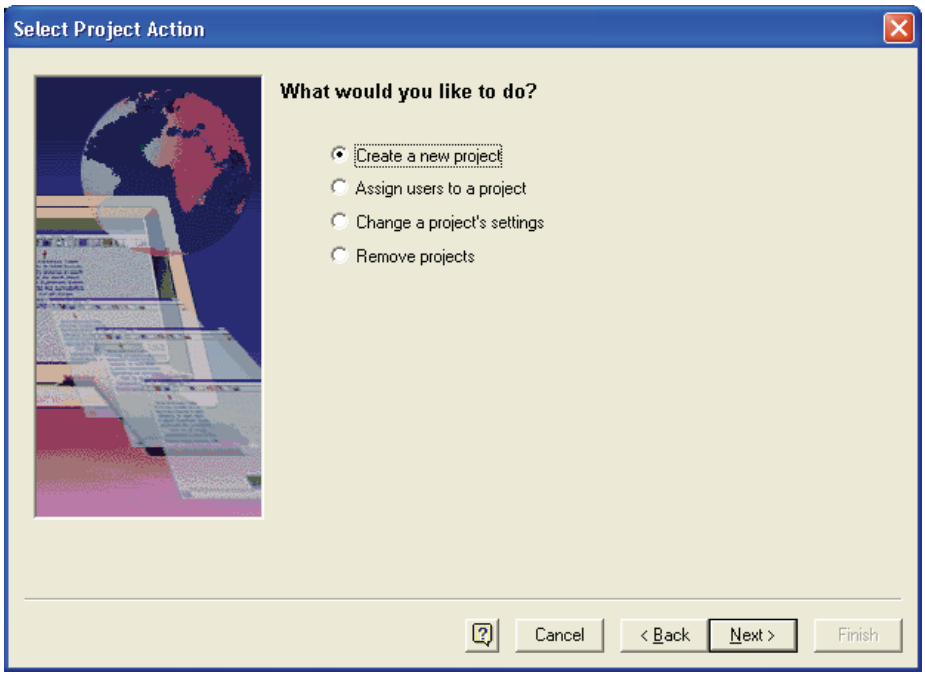
**Figure 38.** Select Administration Wizard page of the System Administration Wizard




3. Click the **Project** option, and then click **Next**.

The Select Project Action page of the Project Wizard appears. See Figure 39.

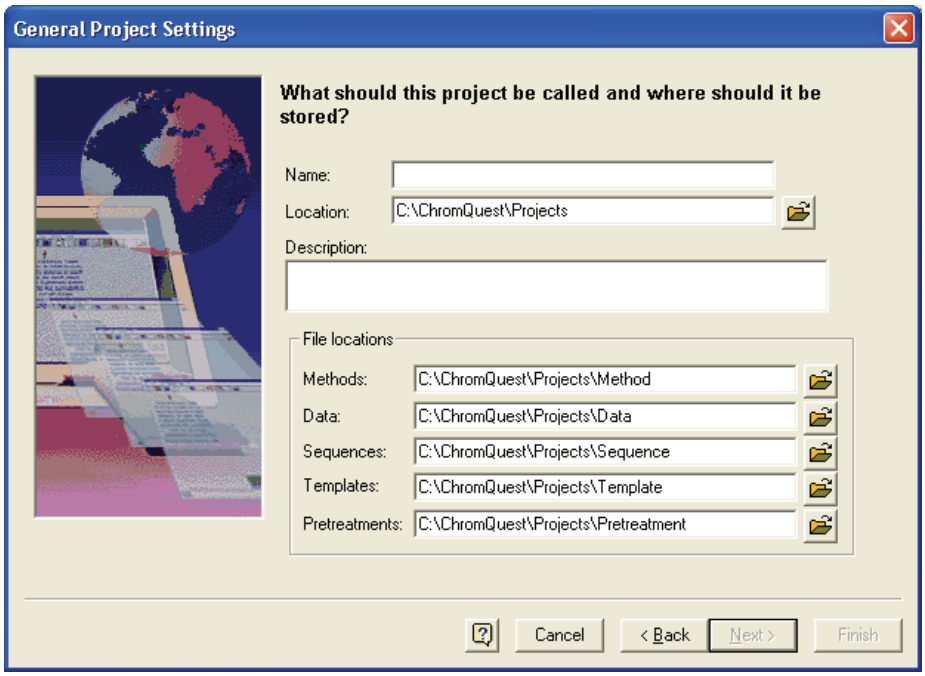
**Figure 39.** Select Project Action page of the Project Wizard



4. Click the **Create A New Project** option, and then click **Next**.

   The General Projects Settings page, where you name the project and set up the file locations, appears. See Figure 40.

**Figure 40.** General Project Settings page of the Project Wizard

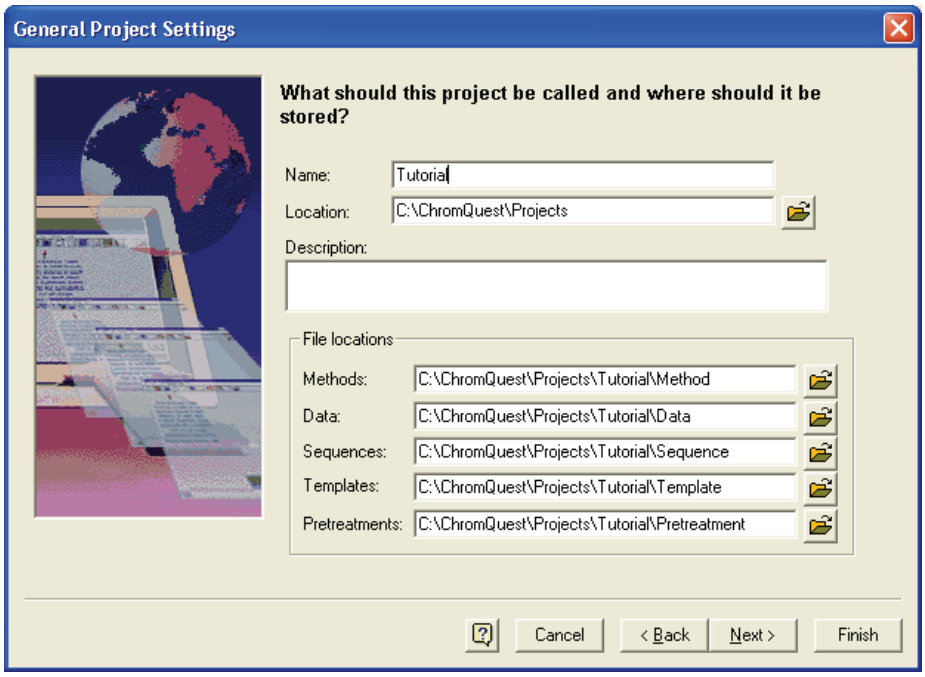



5. Give the project a name:

   a. Type **Tutorial** in the Name box.

      As you type a name in the Name box, the location of the project files updates, as shown in Figure 41.

**Figure 41.** Creating a project name

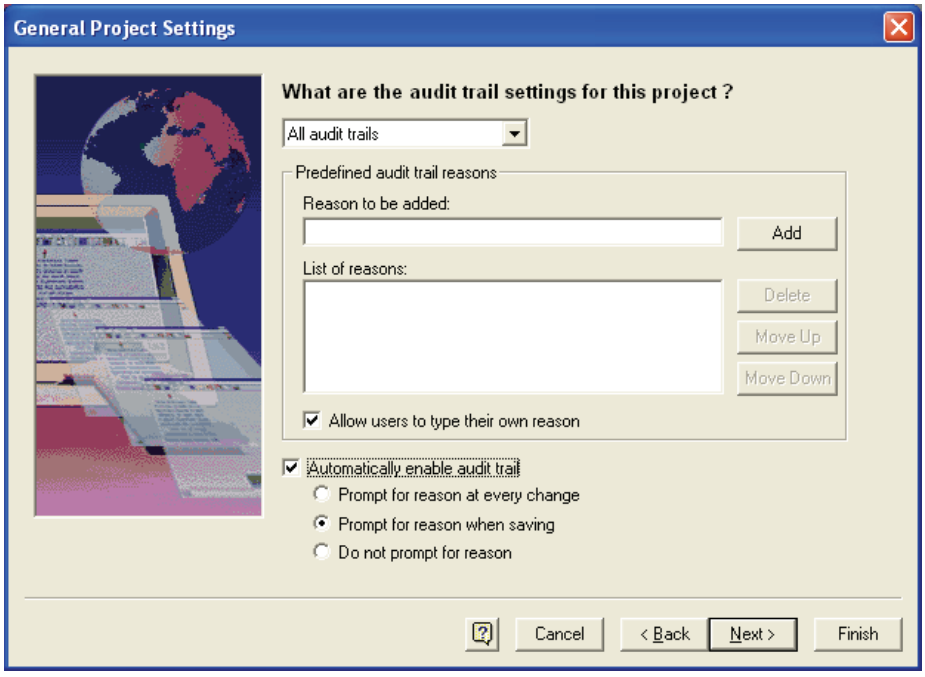



b. (Optional) Type a description of the project in the Description box.

c. Click **Next**.

The second page of the General Project Settings Wizard, where you select the auditing options, appears. See Figure 42.

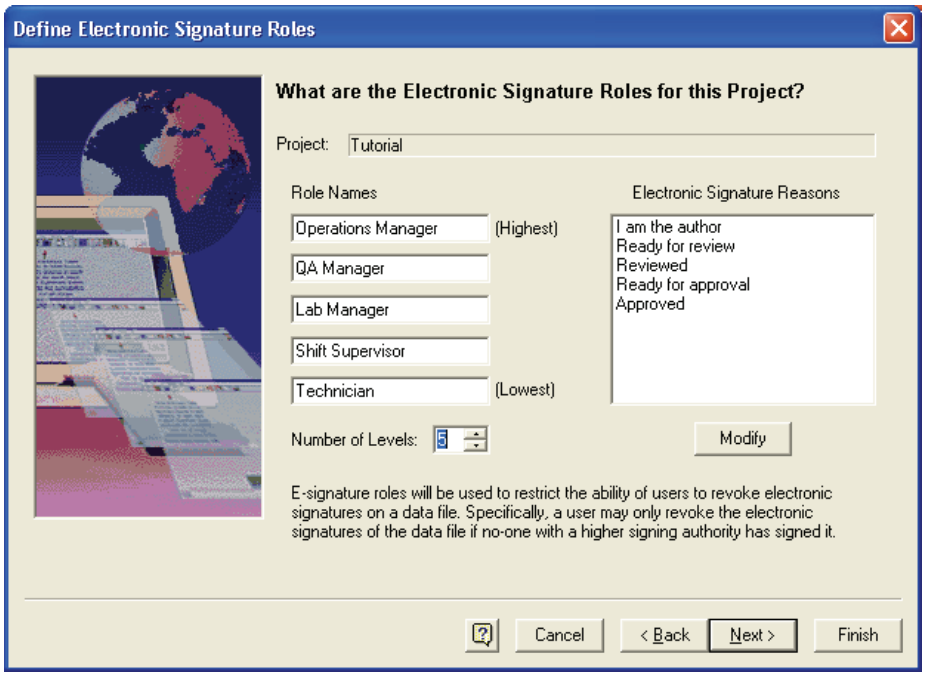
**Figure 42.** Audit trail options page of the Project Wizard



6. Select the audit trail settings for the project:
   a. Under What are the audit trail settings for this project?, select the items to be audited from the list. The available selections are as follows: All audit trails, Advanced report audit trail, Method audit trail, Pretreatment audit trail, or Sequence audit trail.
   b. (Optional) Create a list of predefined audit trail reasons:
      - In the Reason to be added box, type a reason for modifications to be made, and then click **Add**. The predefined reason appears in the List of reasons box.
      - Use the Delete, Move Up, and Move Down buttons to modify the list.
   c. To allow users to type their own reasons, select the **Allow users to type their own reason** check box.
   d. To automatically enable the audit trail, select the **Automatically enable audit trail** check box. Then select the option for when you want the prompt for entering a reason to appear.
   e. Click **Next**.

      The Define Electronic Signature Roles page of the Project Wizard appears. See Figure 43.

**Figure 43.** Define Electronic Signature Roles page of the Project Wizard




7. Define the electronic signature roles for the project:
   a. Type **5** in the Number of Levels combo box.
   b. (Optional) Modify the signature reasons by clicking **Modify** to open the Modify Electronic Signature Reasons dialog box. Add, delete, or modify the signature reasons.

c. Click **Next**.

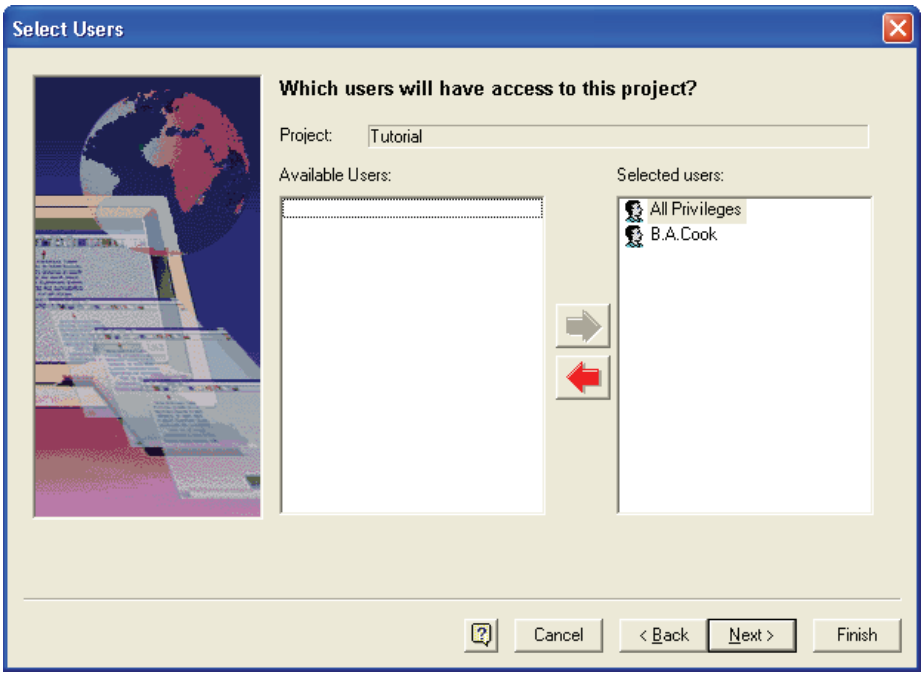
The Select Users page appears.

8. Select the users for the Tutorial project that you are creating:

a. In the Available Users box, double-click each user that you want to add to this project. Add **All Privileges** and yourself (your user name) to the Selected Users box.

After you double-click an available user, the name of the user moves to the Selected Users list. See Figure 44.

**Figure 44.** Select Users page of the Project Wizard




b. Click **Next**.

The Set User Privileges page of the Project Wizard appears. See Figure 45.

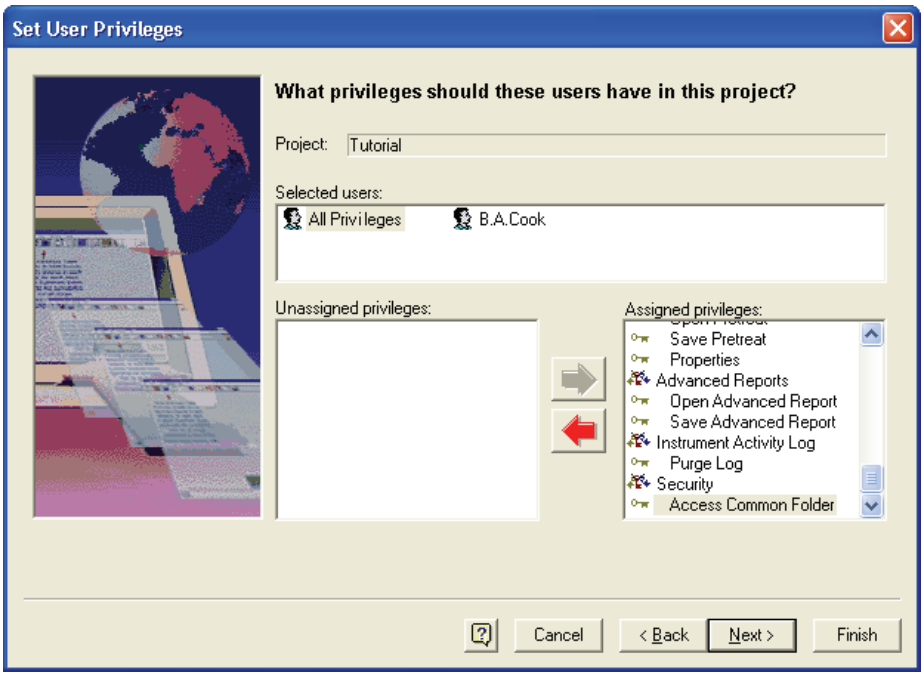
9. Set the privileges for the users of the Tutorial project:

a. Select **All Privileges** and yourself (your user name) in the Selected Users box, and then double-click all the privileges in the Unassigned privileges box.

**Note** You can assign privileges by type, such as giving a user privileges to all the method keys; or you can assign privileges individually, such as letting a user open methods, but not save methods.

b. When you double-click a privilege, it moves to the Assigned Privilege box. See Figure 45.

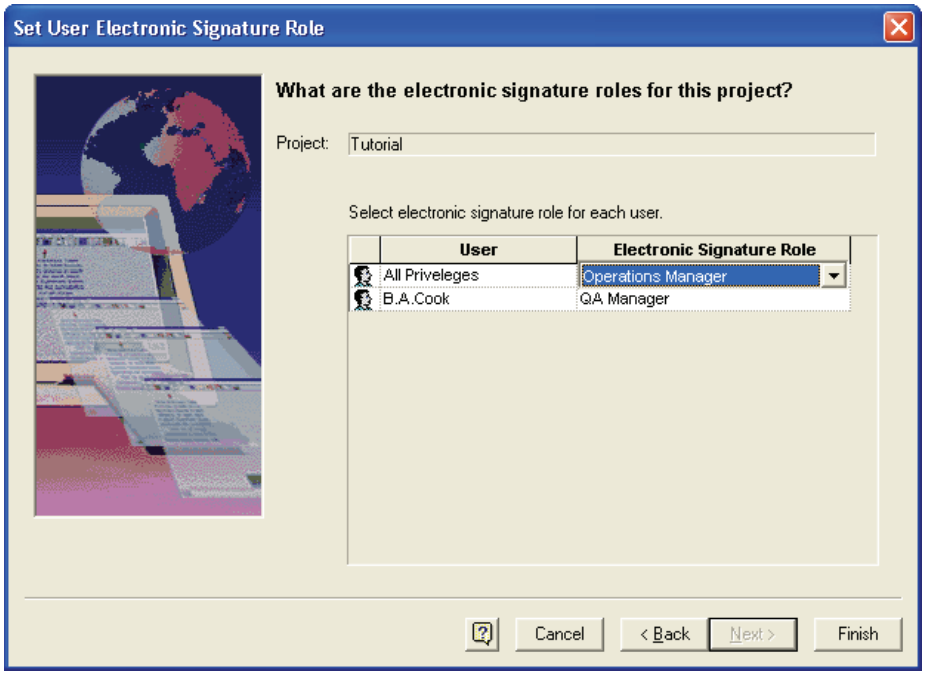
**Figure 45.** Set User Privileges page of the Project Wizard




c. Click **Next**.

The Set User Electronic Signature Role page appears. See Figure 46.

**Figure 46.** Set User Electronic Signature Role page of the Project Wizard




10. Select the electronic signature role for each user with privileges to the Tutorial project, and then click **Finish**. ChromQuest saves the project settings.

# Assigning Privileges to Users

After you define your Enterprise and create users, you are ready to assign privileges to users.

In this tutorial, you assign instrument and system administration privileges to users. You allow users access to every instrument in the Enterprise. You assign access to the existing projects, as well as all privileges within each project.

Users with system administration privileges can do the following:

- Access the Options dialog box – Enterprise page to enable login and project management and create user lists.
- Access the Options dialog box – E-mail page to set up e-mail options.
- Access the Options dialog box – General page, where the Extended Security, the Automatically Enable Method Audit Trail, the Save All Analysis Results, and the Instrument Activity Log Purge Authorized Only After Archive options are located.
- Access the System Administration Wizard to create projects and assign privileges to users.

Users with instrument administration privileges can do the following:

- Add and configure instruments.
- Access the Calibration and Maintenance pages of the Diagnostics dialog box for the Surveyor LC Pump Plus.
- Access the Diagnostics dialog box for the Surveyor Autosampler Plus.

### ❖ To assign privileges to users

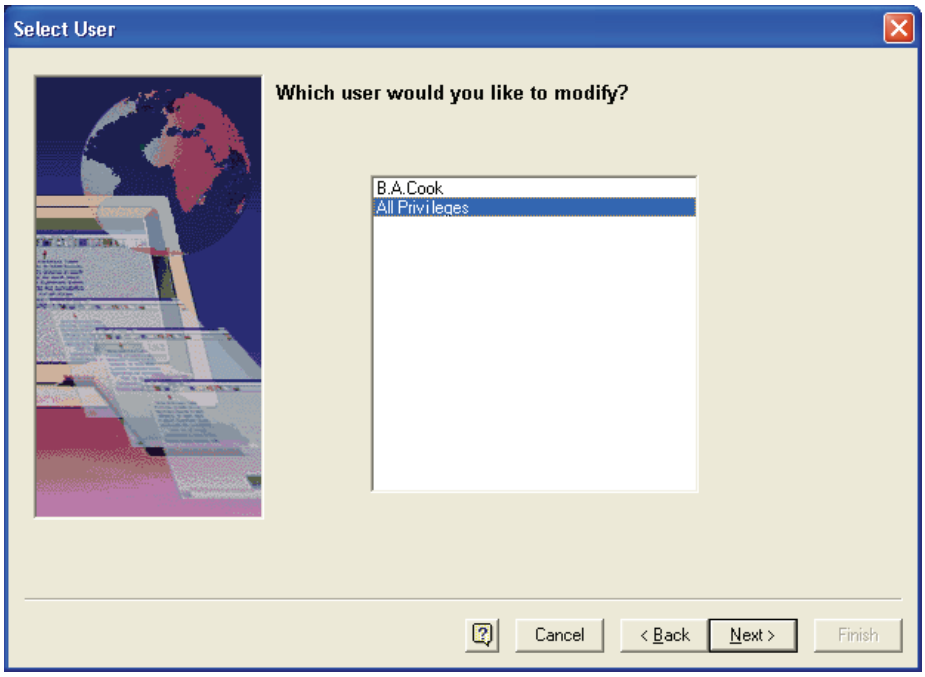
1. Open the Select Administration page of the System Administration Wizard as described in steps 1 to 3 of "Creating a Project" on page 47.
2. In the Select Administration page, do the following:
   a. Click the **User** option.
   b. Select the **Restart Selected Wizard When Finished** check box.

      You can assign user privileges to only one user at a time. Use the Restart Selected Wizard When Finished option to enter privileges for more than one user without having to restart the System Administration Wizard.

   c. Click **Next**.

      The Select User page, shown in Figure 47, appears.

**Figure 47.** Select User page of the User Wizard

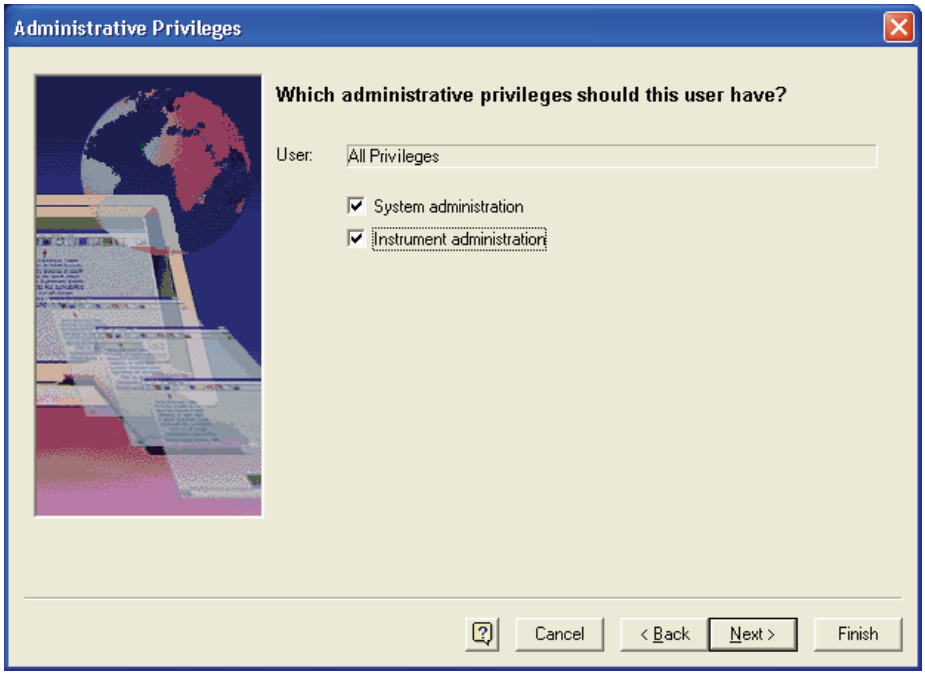



3. Select **All Privileges** or the user that you want to modify, and then click **Next**.

**Note** If the User list is empty, see "Enabling Instrument Login and Project Management" on page 43 for information on adding users.

The Administrative Privileges page appears. See Figure 48.

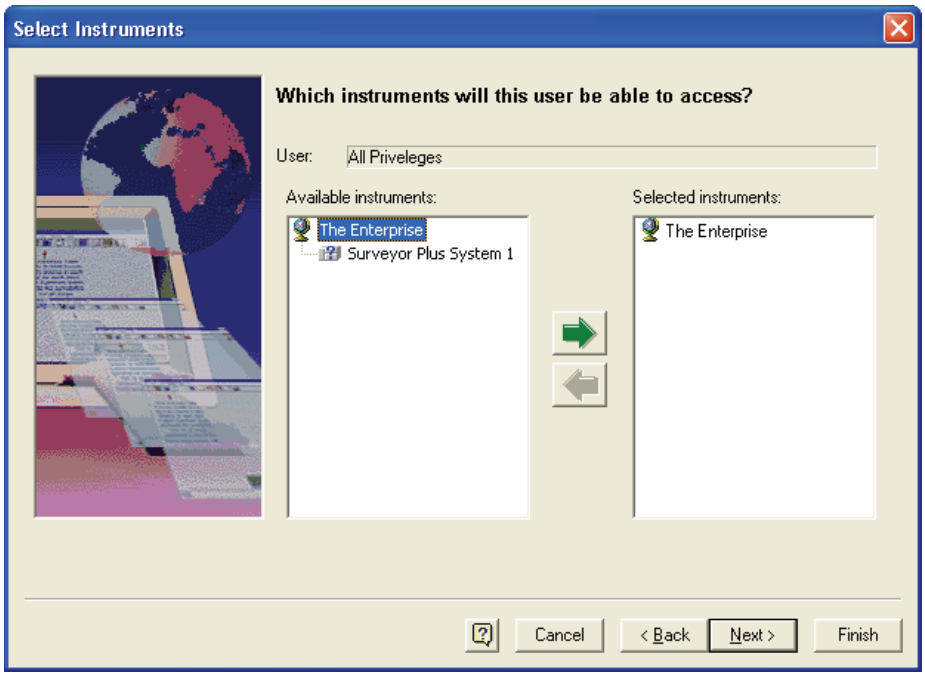
**Figure 48.** Administrative Privileges page of the User Wizard



4. Give the selected user instrument and system administration privileges by selecting the **System Administration** and the **Instrument Administration** check boxes, and then click **Next**.

   The Select Instruments page appears. See Figure 49.

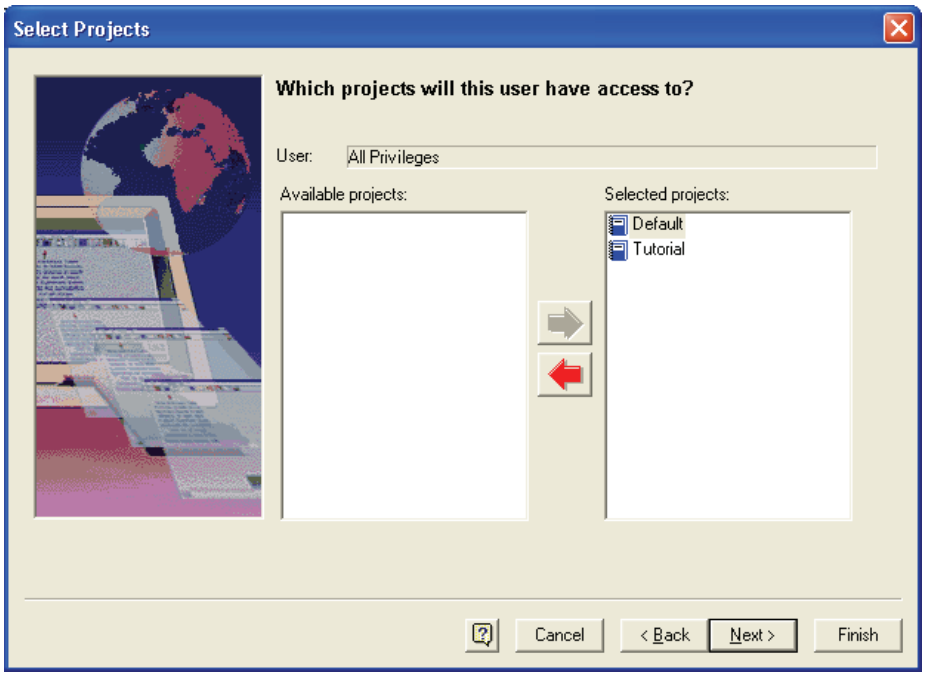
   **Figure 49.** Select Instruments page of the User Wizard




5. Give the user privileges to all the instruments in the Enterprise by double-clicking **The Enterprise** icon, and then click **Next**.

   The Select Projects page appears. See Figure 50.

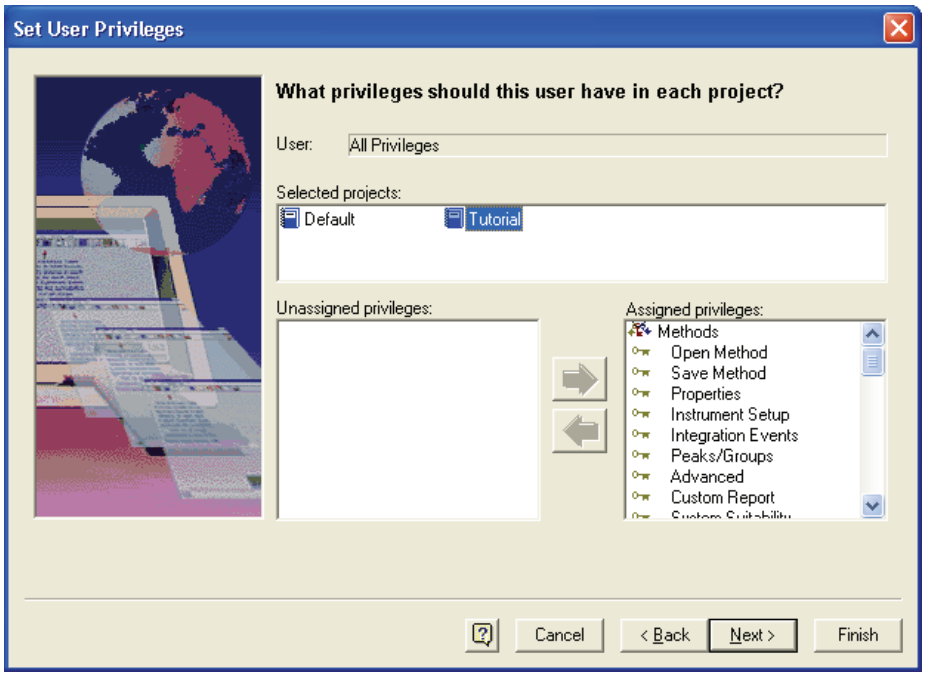
**Figure 50.** Select Projects page of the User Wizard




6. Double-click both the Default project and the Tutorial project in the Available projects box, and then click **Next**.

   The Set User Privileges page appears. See Figure 51.

**Figure 51.** Set User Privileges page of the User Wizard



7. Give the user every privilege in both the Default project and the Tutorial project:

   a. Select **Default** in the Selected projects box, and then double-click the privileges in the Unassigned privileges box.

   **Note** Privileges can be assigned one by one or by file type. Assign privileges one by one by double-clicking the keys. Assign privileges by file type by double-clicking a key set.

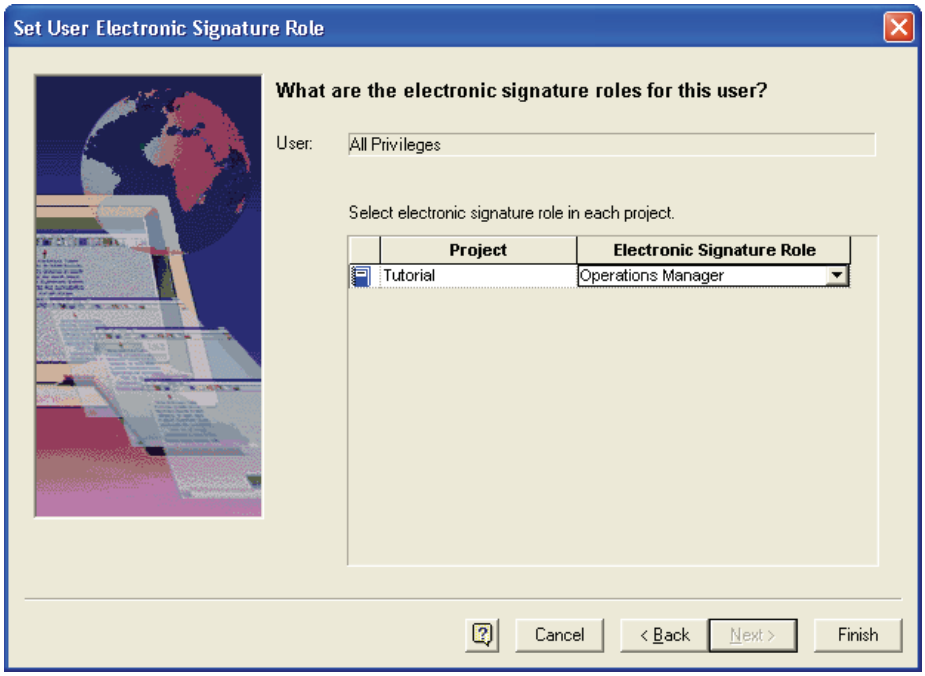
   **Key Sets**
   - Methods
   - Data
   - Sequences
   - Control
   - Pretreatment
   - Advanced Reports
   - Instrument Activity Log

   b. Select **Tutorial** in Selected projects box, and then double-click the privileges in the Unassigned privileges box.

   c. Click **Next**.

   The Set User Electronic Signature Role page appears. See Figure 52.

**Figure 52.** Set User Electronic Signature Role page of the User Wizard



8. Select the electronic signature roles for the user, All Privileges, to both the Default and Tutorial project, and then click **Finish.**

   ChromQuest saves the project, user, and instrument information, and then returns you to the Select User page of the User Wizard.

9. Assign yourself (your user name) Instrument Administration privileges, System Administration privileges, access to the Enterprise, and every privilege for the Default and Tutorial projects.

10. Close the System Administration Wizard.

11. If you do not want to enable the security and administration features offered in ChromQuest, do the following:

    a. From the Main Menu bar, choose **Tools > Options**.

    b. Click the **Enterprise** tab.

    c. Clear the **Enable instrument login and project management** check box.

# Configuring Your Instrument

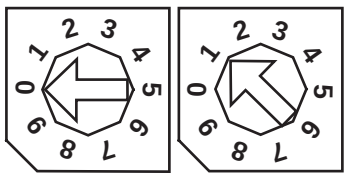
In this tutorial, you learn how to add an instrument to the Enterprise. The Enterprise includes all instruments controlled within a domain or all instruments controlled by a standalone workstation. In addition, you learn how to configure your instrument so that the data system recognizes it.

**Note** ChromQuest contains a login security feature that allows the system administrator to assign privileges to users and groups. If this security feature has been enabled, you must have instrument privileges to be able to add an instrument to the Enterprise or to configure an instrument.

**Note** ChromQuest SI controls one instrument and does not provide security features.

Before you configure your instrument, look at the back panels of its modules. On the back panel of each module, you will find a unit ID that consists of two rotary switches. See Figure 53. Each rotary switch contains ten positions. The arrow on the left switch points to the "tens" digit of the unit ID. The arrow on the right switch points to the "ones" digit of the unit ID. The unit ID can range from 01 to 99. The value 00 is reserved for service functions. When you configure a module, you enter this unit ID value in the Stack ID boxes. The unit ID value must match the Stack address or the module will not communicate with the data system.

**Figure 53.** Unit ID set to 01




**Contents**

# Adding an Instrument to the Enterprise

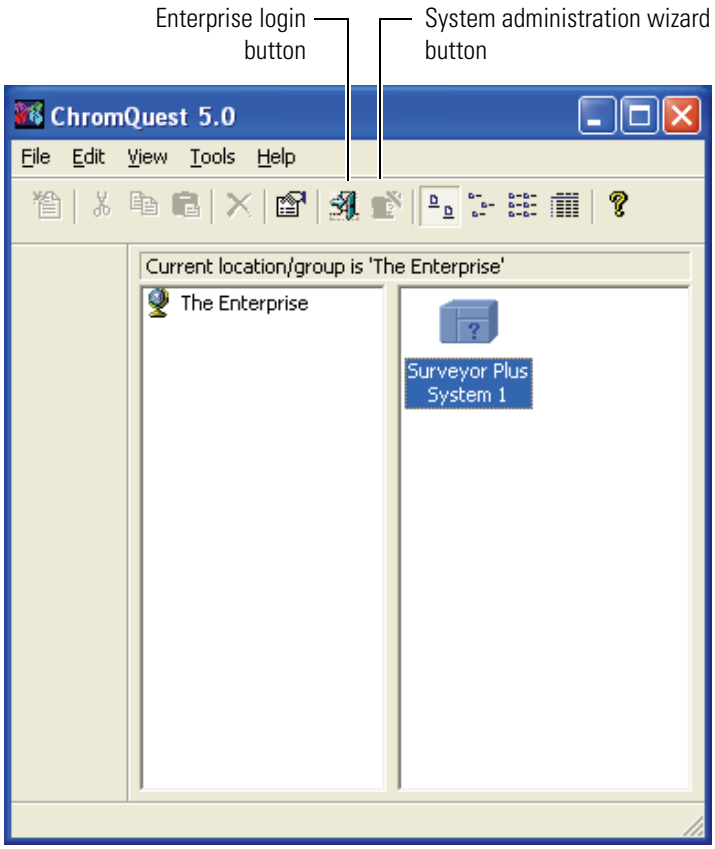
In ChromQuest, the Enterprise consists of one or more networked instruments.

If you are controlling your Surveyor instrument from ChromQuest SI, skip this topic.

### ❖ To add a new instrument to the Enterprise

1. Double-click the ChromQuest icon on the Windows desktop to open the Main Menu window (see Figure 54). Alternatively, choose **Start > Programs > Chromatography > ChromQuest** to open ChromQuest.

   **Figure 54.** Main Menu window with System Administration locked




2. Look at the toolbar in the Main Menu window.


Locked


Unlocked

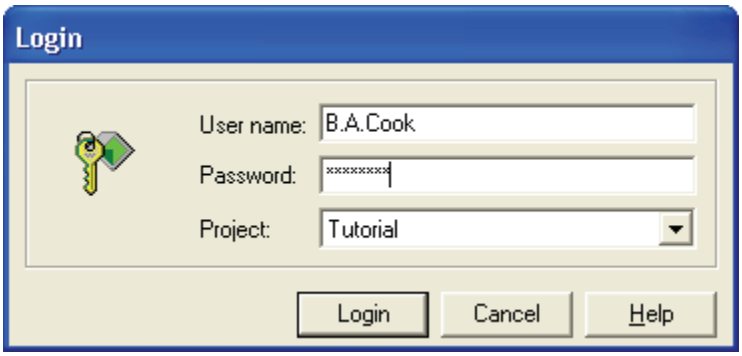
   - If the System Administration Wizard button is unlocked, continue at step 4.
   - If the System Administration Wizard button is locked, the system administrator has enabled the security feature, and you are required to login. Continue at step 3.

3. To login to the ChromQuest chromatography data system:

   a. Click the **Enterprise Login or Logout** button.

      The Login dialog box, shown in Figure 55, appears.

**Figure 55.** Enterprise Login dialog box




b. Type your user name in the User Name box.

c. Type your password in the Password box.

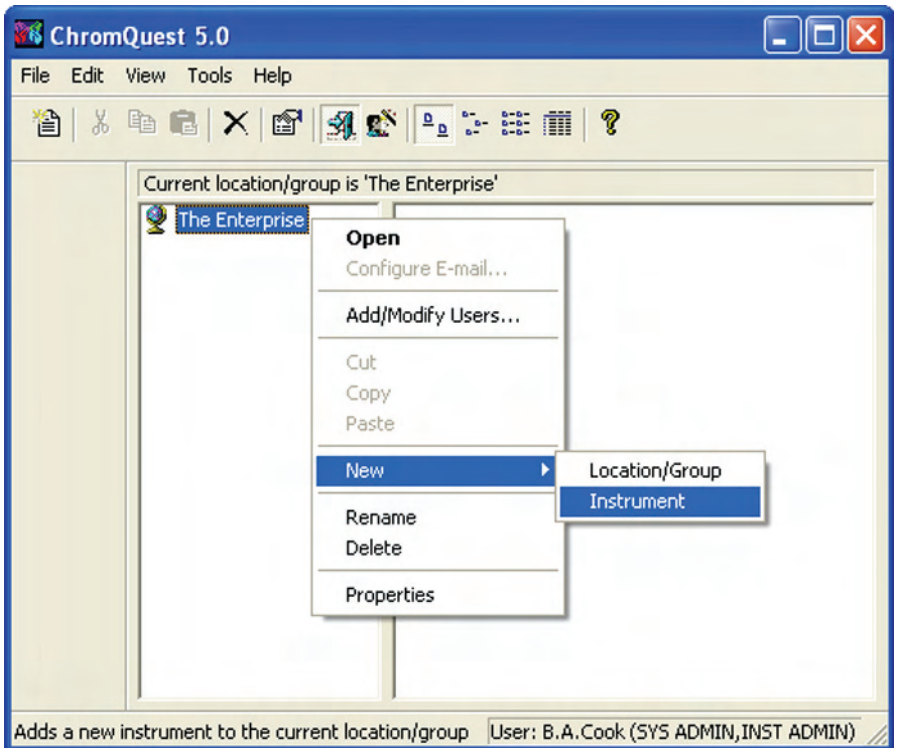
d. If available, select the appropriate domain from the Domain list.

**Note** If your workstation is not networked to a domain controller, the Enable Administration login box does not contain the Domain list.

e. Click **Login**.

4. Right-click **The Enterprise** icon, which is located on the left panel of the Main Menu window, to open a shortcut menu.

**Figure 56.** Enterprise shortcut menu



5. Choose **New > Instrument** from the shortcut menu.

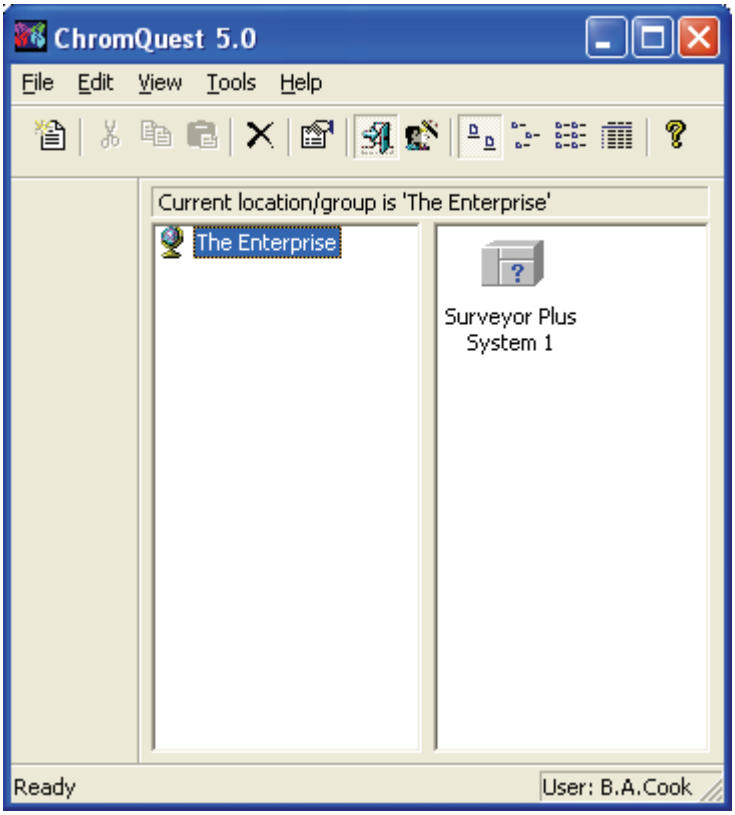
   A new instrument icon with a question mark appears in the right pane.

6. Type a new name for the instrument (for example, Surveyor Plus System 1) in the box below the instrument button, and then press ENTER.

Now that you have created a new instrument, you are ready to configure its modules.

**Note** The Enterprise can be further subdivided into locations and groups. If your Enterprise consists of locations and groups, add your new instrument to the appropriate location or group.

**Figure 57.** Main window for ChromQuest 4.2, showing the newly created instrument



# Configuring Your Instrument

You must configure each module of the instrument.

❖ **To configure the instrument**

1. Opening the Surveyor Modules Dialog Box
2. Adding Modules to the Instrument Configuration
3. Completing the Configuration of Each Module
4. Selecting the Baseline Check Option
5. Returning to the Main Menu Window

**Note** If you are changing the configuration of an instrument, you must exit the Instrument window before you open the Instrument Configuration dialog box.

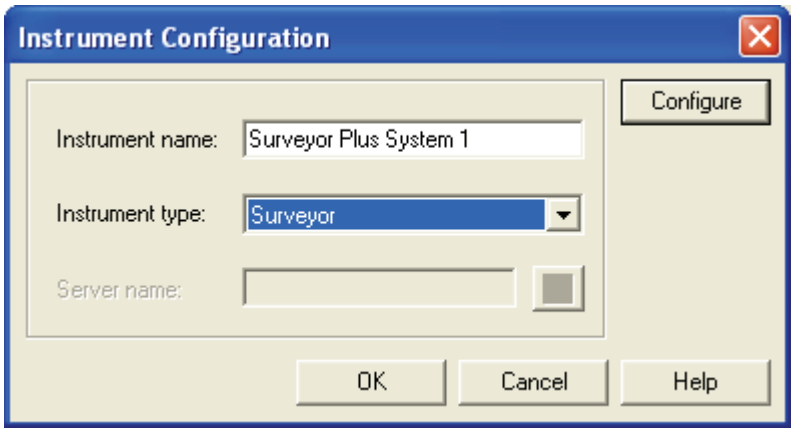
## Opening the Surveyor Modules Dialog Box

❖ **To open the Surveyor Modules dialog box**

1. Do one of the following:
   - For ChromQuest, in the Main Menu window, right-click the icon of the instrument that you want to configure, and then choose **Configure > Instrument** from the shortcut menu.
   - For ChromQuest SI, choose **Start > All Programs > Chromatography > ChromQuest SI Config**. The ChromQuest SI Configuration dialog box appears. Click **Instrument Configuration**.

   The Instrument Configuration dialog box appears. See Figure 58.

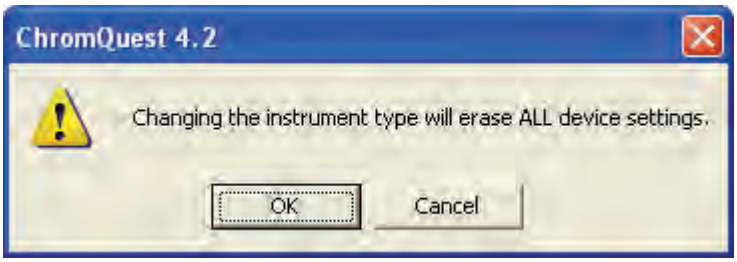
**Figure 58.** Instrument Configuration dialog box



2. In the Instrument Configuration dialog box (see Figure 58), do one of the following:

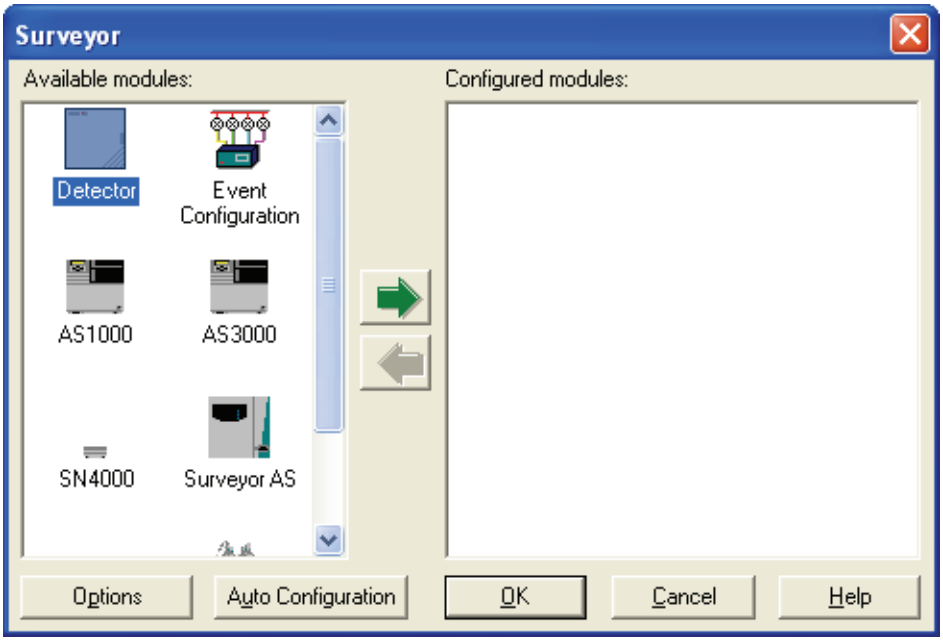
   - To erase the current configuration or change the instrument type, select **Surveyor** from the Instrument type list, and then click **OK** in the dialog box that appears. See Figure 59.

     **Figure 59.** Warning message dialog box

   - To modify your current instrument configuration, click **Configure** in the Instrument Configuration dialog box (see Figure 58) to open the Surveyor dialog box (see Figure 60).

     **Figure 60.** Surveyor dialog box

## Adding Modules to the Instrument Configuration

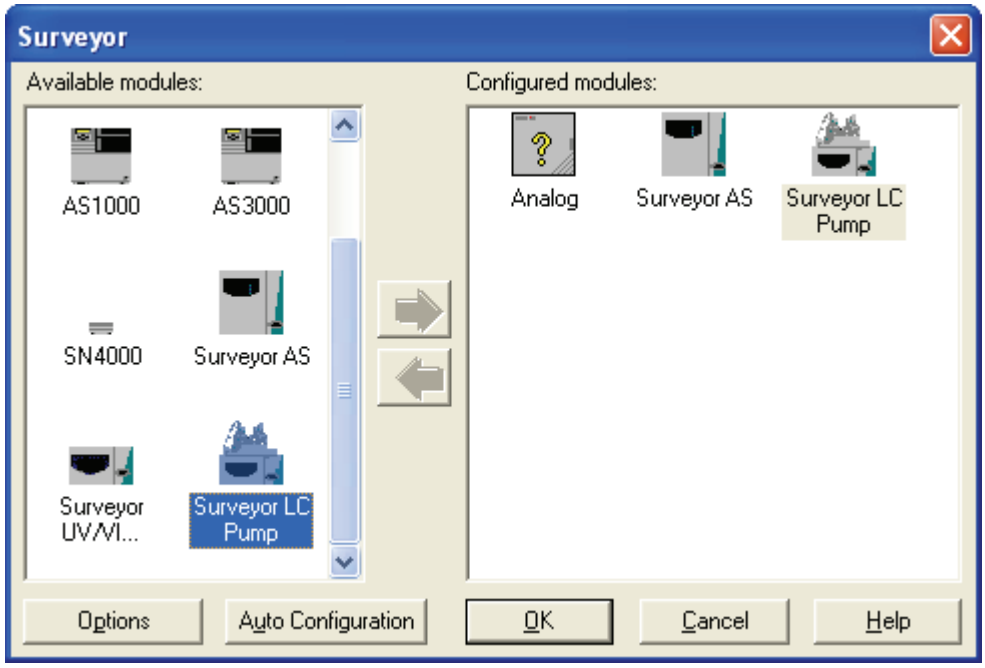
Now that you have the Surveyor dialog box open (see Figure 60), you are ready to add the modules of your instrument to its configuration. You can add the modules in any order.

❖ **To add the instrument modules to the Configured Modules box**

- Double-click the **Surveyor LC Pump** button to add the pump to the Configured modules box.
- Double-click the **Surveyor AS** button to add the autosampler to the Configured modules box.
- If you have a Surveyor UV/Vis Plus Detector, a Surveyor FL Plus Detector, or a Surveyor RI Plus Detector, double-click its respective button to add it to the Configured modules box.
- If you have a Surveyor PDA Plus Detector, double-click the **Detector** button to begin the process of adding the Surveyor PDA Plus Detector to the configuration.

  As you double-click a button in the Available modules box, it moves to the Configured modules box, as shown in Figure 61. The Detector button defaults to the Analog icon in the Configured modules box (see Figure 61).

**Figure 61.** Surveyor dialog box, showing added modules



# Completing the Configuration of Each Module

Now that you have added the modules of your instrument to the Configured modules box, you are ready to complete their configuration.

This topic contains the following procedures. Perform the procedures that pertain to your Surveyor Plus system.

- Configuring the LC Pump
- Configuring the Autosampler
- Configuring the PDA Detector
- Configuring the UV/Vis Detector
- Configuring the FL Detector
- Configuring the RI Detector

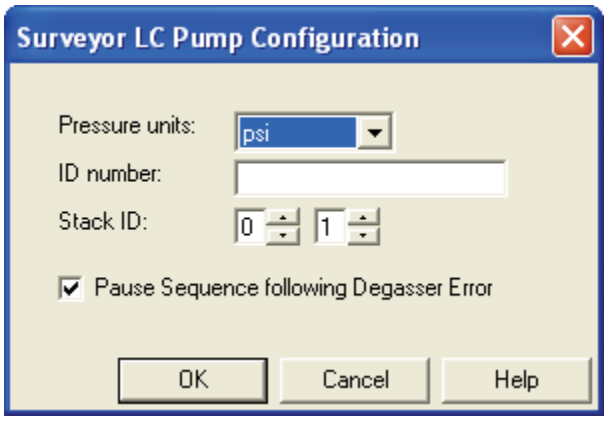
## Configuring the LC Pump

❖ **To complete the configuration of the Surveyor LC Pump Plus**

1. Double-click the **Surveyor LC Pump** icon in the Configured modules box (see Figure 61).

   The Surveyor LC Pump Configuration dialog box, shown in Figure 62, appears.

   **Figure 62.** Surveyor LC Pump Configuration dialog box




2. From the Pressure list, select the Pressure units—**MPa**, **Bar**, or **psi**—that you prefer to use to display the backpressure of your system.

   1 MPa = 10 Bar = 145 psi.

3. (Optional) Type an identification number for the pump in the ID Number box.

4. In the Stack ID box, type or select the unit ID value for the pump. See Figure 53. Unless you are operating more than one Surveyor LC Pump Plus from the same computer, do not change the unit ID value (rotary switch settings on the back panel of the pump). The rotary switches are set to 01 at the factory.
5. If you want the injection sequence to pause if the built-in degasser loses vacuum, select the **Pause Sequence following Degasser Error** check box.

## Configuring the Autosampler

### ❖ To configure the Surveyor.Autosampler Plus

1. Double-click the **Surveyor Autosampler** icon in the Configured modules box.

   The Surveyor Autosampler Configuration dialog box appears.
2. Set the parameters in the dialog box to the settings that are shown below and are reflected in Figure 63.

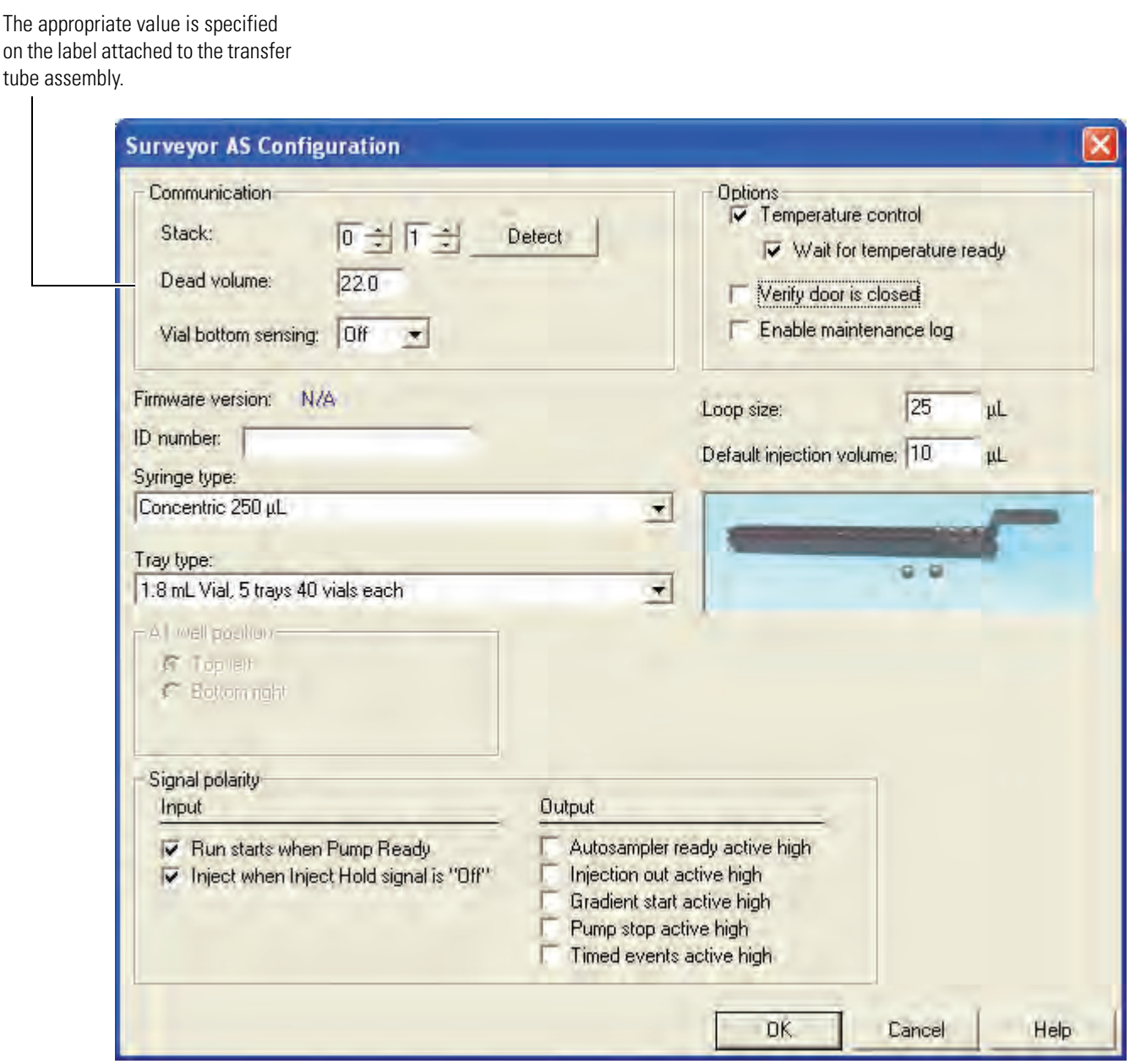
| Parameter | Setting | Result |
|---|---|---|
| Communication | | |
| • Stack | The value of the Unit ID on the back panel of the autosampler. | Must match the Unit ID on the back panel of the autosampler. |
| • Dead volume | The value specified on the transfer tube assembly label. | Specifies the volume of the transfer tubing that connects the injection port to the Valco injection valve. See Figure 14. |
| Options | | |
| • Verify door is closed | ☐ | Specifies that the autosampler can begin an injection or that direct control commands can be performed whether the tray compartment door is open or closed. |
| • Loop size | Enter the size of the sample loop. | Specifies the injection volume for the Full Loop injection mode. |
| • Syringe type | Select the appropriate type for your syringe. | Specifies the type of syringe. The autosampler ships with a 250 µL concentric syringe. |
| Signal Polarities | | |
| • Run starts when Pump Ready | ☑ | Specifies that the pump sends a signal to the autosampler while it detects a stable backpressure. |
| • Inject when Inject Hold signal is Off | ☑ | Specifies that the pump sends a signal to the autosampler when it reaches a certain point in its piston cycle. |

3. Under Communication, click **Detect**.

   There are two Surveyor Autosampler Plus models: the full-featured Surveyor Autosampler Plus and the Surveyor Autosampler Lite Plus. The Surveyor Autosampler Lite Plus does not come with a built-in column oven or tray temperature control. Clicking the Detect button downloads the autosampler model to the ChromQuest data system.

4. Click **OK** to exit the Surveyor Autosampler Configuration dialog box.

**Figure 63.** Surveyor AS Configuration dialog box, showing configuration settings



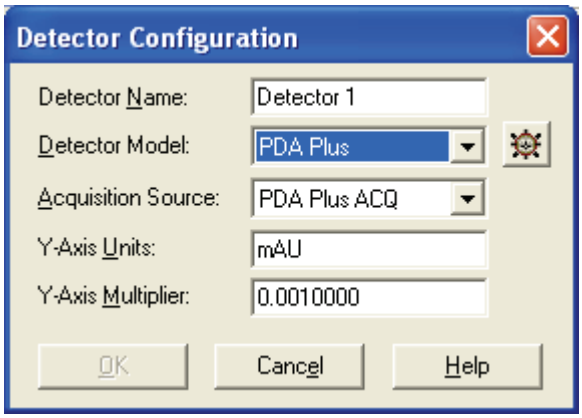
## Configuring the PDA Detector

### ❖ To complete the configuration of the Surveyor PDA Plus Detector

1. Double-click the **Analog** icon in the Configured modules box.

   The Detector Configuration dialog box, shown in Figure 64, appears.

**Figure 64.** Detector Configuration dialog box



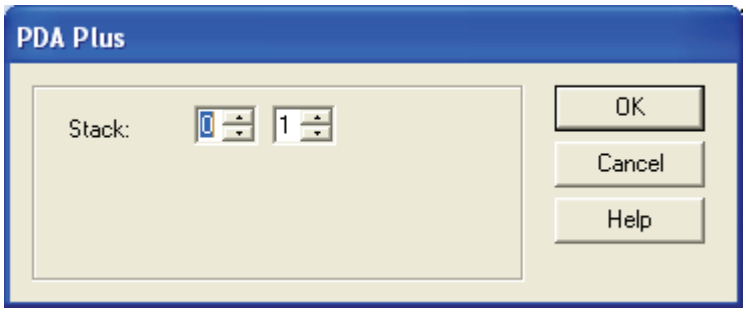

2. In the Detector Model list, select **PDA Plus**.

> **Note** After you select PDA Plus, the Acquisition Source list automatically lists the PDA ACQ option. Leave the Y-axis Units set to mAU and the Y-axis Multiplier set to 0.001. ChromQuest stores the absorbance data from the PDA in microvolts. The Y-axis Multiplier of 0.001 scales the display to mV (1 mV = 1 mAU).

3. Click the button to the right of the Detector Model list.

   The PDA Plus dialog box, shown in Figure 65, appears.

**Figure 65.** PDA Plus dialog box




4. In the Stack boxes, select or type the unit ID value for your Surveyor PDA Plus Detector.
5. Click **OK** to exit the PDA Plus dialog box.

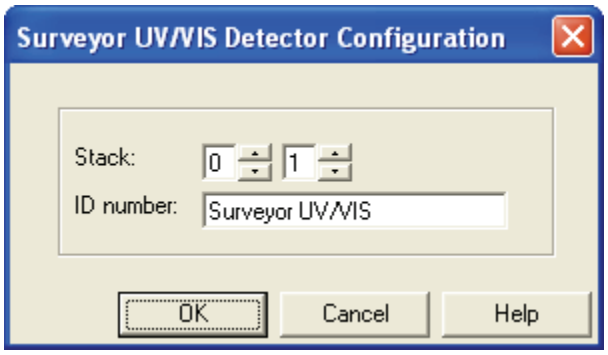
## Configuring the UV/Vis Detector

### ❖ To complete the configuration of the Surveyor UV/Vis Plus Detector

1. Double-click the **Surveyor UV/VIS** icon in the Configured modules box.

   The Surveyor UV/VIS Detector Configuration dialog box, shown in Figure 66, appears.

   **Figure 66.** Surveyor UV/Vis Detector Configuration dialog box




2. In the Stack boxes, type or select the unit ID value for your detector.

   The unit ID on the back panel of the detector consists of two rotary switches. Each switch has ten positions. The arrow on the left switch points to the "tens" digit of the unit ID. The arrow on the right switch points to the "ones" digit of the unit ID.

3. (Optional) Type an identification number for your Surveyor UV/VIS Detector, such as its license number, in the ID number box.

4. Click **OK** to exit the Surveyor UV/VIS Detector Configuration dialog box.

## Configuring the FL Detector

### ❖ To complete the configuration of the Surveyor FL Plus Detector

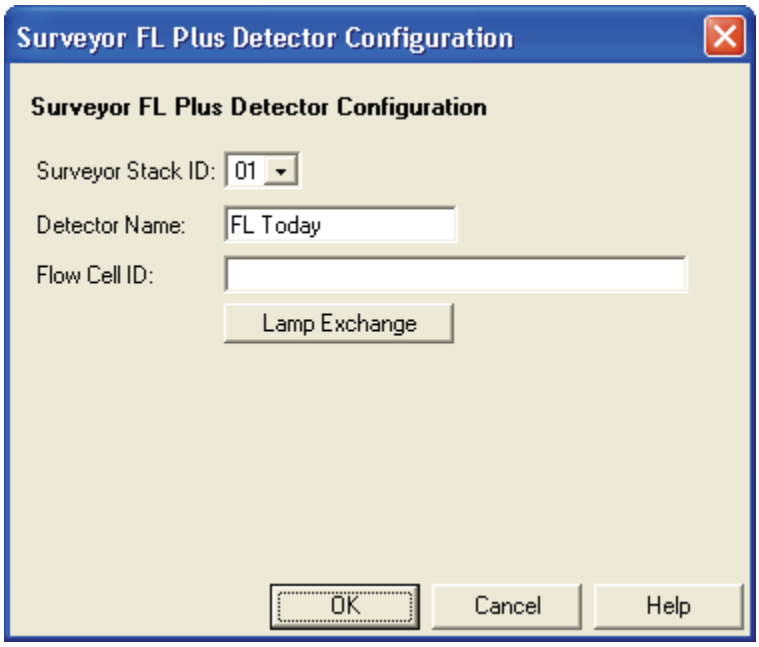
1. Double-click the **Surveyor FL Plus Detector** icon in the Configured modules box.

   The Surveyor FL Plus Detector Configuration dialog box, shown in Figure 67, appears.

2. (Optional) Type an identifying name in the Detector Name box.

3. (Optional) Type an identifying label in the Flow Cell ID box.

**Figure 67.** Surveyor FL Plus Detector Configuration dialog box



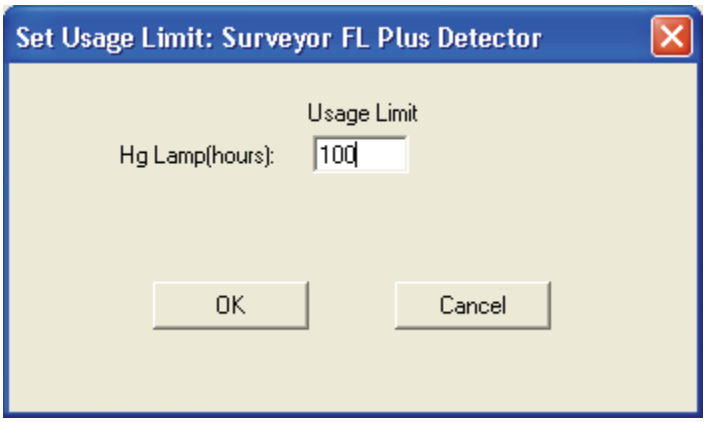

**Note** The Surveyor FL Plus Detector uses the light spectrum produced by a Hg lamp to calibrate its wavelength accuracy. This lamp is turned on only during a wavelength calibration.

4. To set a usage limit for the Hg lamp:

   a. Click **Lamp Exchange**.

   The Set Usage Limit: Surveyor FL Plus Detector dialog box, shown in Figure 68, appears.

   **Figure 68.** Set Usage Limit: Surveyor FL Plus Detector dialog box



b. Type a value for the usage limit in the Usage limit box.

The allowable range of values is 1 to 9999 with a default of 100. The FL detector turns on the mercury lamp on for the Wavelength Check procedure performed by the Maintenance software. The operating life of the mercury lamp is approximately 300 hours.

c. Click **OK** to exit the Set Usage Limit: Surveyor FL Plus Detector dialog box.

d. Click **OK** to exit the Surveyor FL Plus Detector Configuration dialog box.

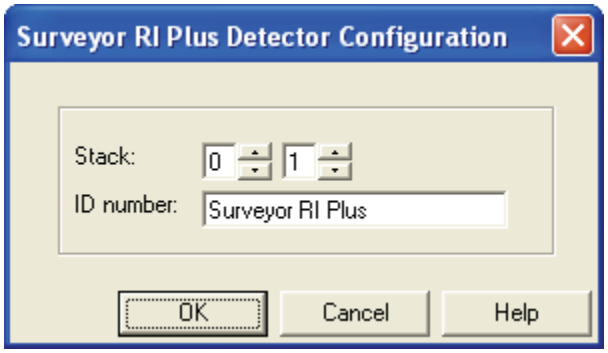
## Configuring the RI Detector

❖ **To complete the configuration of the Surveyor RI Plus Detector**

1. Double-click the **Surveyor RI Plus Detector** button in the Configured Modules box.

   The Surveyor RI Plus Detector Configuration dialog box, shown in Figure 69, appears.

   **Figure 69.** Surveyor RI Plus Detector Configuration dialog box




2. Unless you are controlling more than one Surveyor RI Plus Detector from the same computer, leave the stack value at its default of 1.

   **Note** The Surveyor RI Plus Detector stores its IP address electronically. The detector does not have rotary switches on its back panel.

3. (Optional) Type an identification number in the ID number box.

4. Click **OK**.

## Selecting the Baseline Check Option

ChromQuest 4.2 contains two features that provide information on the stability of the chromatographic baseline: Preview Run and Baseline Check. You can use the Preview Run feature to visually check the baseline. In addition to a visual check, the Baseline Check feature gives you quantitative results for the noise and drift levels of the baseline.

**Note** If you create a method for an instrument that has the Baseline Check feature enabled, you will not be able to use the method for instruments that do not have the Baseline Check feature enabled. The reverse is also true.

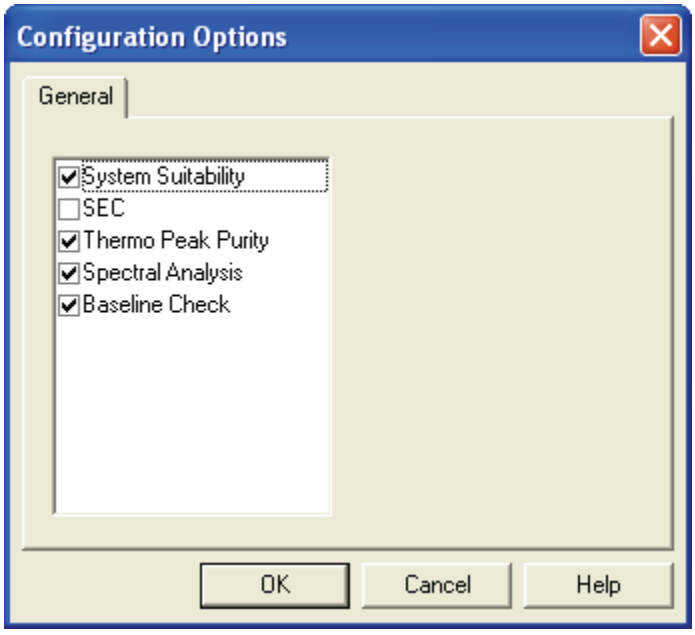
By default, the Baseline Check feature is enabled.

### ❖ To ensure that the Baseline Check feature is enabled

1. In the Surveyor dialog box (see Figure 60), click **Options**.

   The Configuration Options dialog box, shown in Figure 70, appears.

   **Figure 70.** Configuration Options dialog box – General page




2. Verify that the **Baseline Check** check box is selected.
3. Click **OK** to exit the Configuration Options dialog box.

**Note** If you have configured a PDA detector, the Spectral Analysis check box is automatically selected after you exit the Surveyor dialog box.

## Returning to the Main Menu Window

After you finish configuring your instrument, return to the Main Menu window in ChromQuest.

If you are controlling your instrument with the ChromQuest SI data system, close the Instrument Configuration program.

**Note** ChromQuest SI does not contain a Main Menu window.

### ❖ To return to the Main Menu window

1. Click **OK** at the bottom of the Surveyor Modules dialog box (see Figure 61).
2. Click **OK** at the bottom of the Instrument Configuration dialog box (see Figure 58) to return to the Main Menu of ChromQuest.

You have now successfully added and configured the modules of your instrument. In addition, you have verified that the Baseline Check option is selected.

# Creating Methods

In this tutorial, you learn how to create an acquisition method containing the instrument control parameters and column performance information for your chromatographic method. In addition, you learn how to create a shutdown method that turns off the lamps and the mobile phase flow at the end of a sequence run.

**Note** To turn off the lamp in the Surveyor RI Plus Detector, you must turn off the power to the detector.

Before you can perform this tutorial, your instrument must be configured. If your instrument has not been configured, see Chapter 3, "Configuring Your Instrument."

**Contents**



**Note** ChromQuest contains a login security feature that allows the system administrator to assign privileges to users and groups. If this security feature is enabled, your system administrator can restrict your access to specific tasks such as creating or modifying methods. To avoid confusion, determine your user privileges before you begin this tutorial.

# Description of ChromQuest Methods

There are three types of methods in ChromQuest:

- Acquisition methods, which contain the information required to control the instrument modules, acquire data, and process data files
- Shutdown methods, which turn off the pump flow, the detector lamps, or both at the end of a sequence run
- Prep only methods, which contain the information to perform an automated sample preparation routine and maintain a set of chromatographic conditions, such as column temperature and mobile phase parameters

In this tutorial, you create an acquisition method containing the instrument control parameters required to create the specified chromatographic conditions. In addition, you add the column parameters to the method, which enables ChromQuest to calculate the performance parameters for your chromatographic separation.

If your instrument includes a PDA or UV/Vis detector, use the Autosampler Test Mix (P/N A4991-010) contained in the Surveyor Autosampler accessory kit. This test mix consists of a solution of 0.5% toluene in methanol.

If you do not have a vial of the Autosampler Test Mix or you are performing the tutorial to familiarize yourself with the features of the RI or FL detectors, plan to inject an analyte about which you have some chromatographic knowledge and adjust the instrument parameters accordingly.

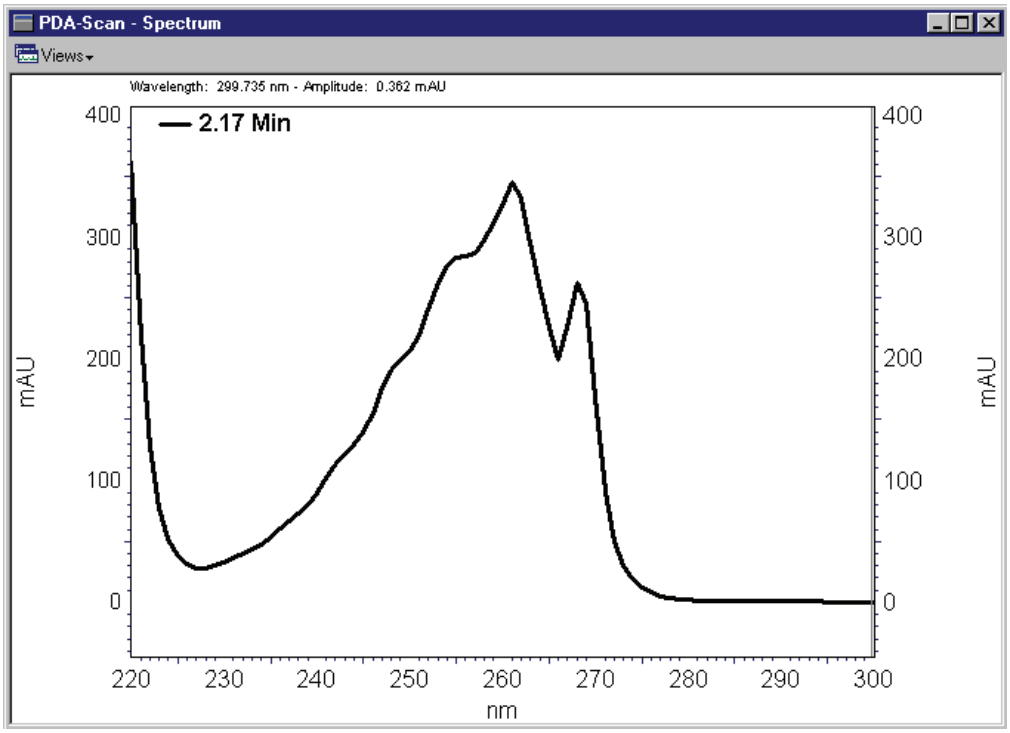
Using the chromatographic conditions listed in Table 7, toluene elutes at approximately 2 minutes.

**Table 7.** Chromatographic conditions for toluene

| Parameter | Setting |
|---|---|
| Mobile phase | 80:20 Methanol \ Water |
| Flow rate | 1 mL/min |
| Column | 100 × 4.6 mm, C-18 column, 5 micron particle size, or equivalent |
| Temperature | ambient |
| Discrete channel wavelengths | • 230 nm, 1 nm bandwidth for the PDA detector<br>• 260 nm, 1 nm bandwidth for the PDA detector |
| Scan range (PDA) | 220 nm to 300 nm, 1 nm step, 1 nm bandwidth |

A spectrum of toluene in 80:20 methanol / water is shown in Figure 71.

**Figure 71.** Spectrum of Toluene in 80:20 methanol / water




If your system contains a Surveyor FL Plus Detector or a Surveyor RI Plus Detector, inject an analyte for which you have a proven chromatographic method. In this tutorial, a solution of anthracene is used for systems containing an FL detector, and a solution of sucrose in water is used for systems contains an RI detector.

The sections of an acquisition method, as well as their descriptions are listed in Table 8. The first section, Instrument Setup, is the only section that you need to complete before you can make an injection. You can complete the other sections of the method after you make an injection and acquire a data file.

**Table 8.** Description of the sections of an acquisition method (Sheet 1 of 2)

| Section | Description |
|---|---|
| Instrument Setup | Use this window to do the following:<br>• Enter the control parameters for each module in your instrument<br>• Add an auxiliary trace to record the backpressure of your system during a run<br>• Enter baseline check parameters if the Baseline Check option in the Configuration Options dialog box is selected<br>• Select a type of trigger |
| Integration Events | This window contains the integration table(s) for your method. There is one integration table for each discrete wavelength and each multi-chromatogram wavelength in the method.<br><br>Use the integration tables to enter the integration parameters for each discrete or multi-chromatogram wavelength specified in the method. To open a specified integration table, select the wavelength in the Analysis Channel list. |
| Spectral Options | This window is available if your system contains a configured PDA detector. This window contains the following pages: Library, Purity, Spectrum, Multi-Chromatogram, and Ratio.<br><br>Use this window to do the following:<br>• Select the automated Library search parameters<br>• Select the parameters for peak purity calculations<br>• Select spectral filtering and spectral definition options<br>• Select multi-chromatogram wavelengths<br>• Set up ratio plots |
| Peak/Groups | Use the Peak table to enter the calibration curve parameters for your analytes. Use the Group tables to combine peaks into groups and to calculate group totals. There is one Peak table for each configured detector of your instrument. |
| Review Calibration | Use this window to review the calibration curve for your method. This window displays the calibration table, a graphical display of the calibration points, the calibration parameters, and the regression statistics for each named peak in the Peak table. |
| Advanced | This window contains the following pages: Export, Custom Parameters, Column / Performance, Files, and Advanced Reports.<br><br>Use this window to calculate performance parameters such as capacity factor and resolution, column parameters must be entered in the Column / Performance page. |

**Table 8.** Description of the sections of an acquisition method (Sheet 2 of 2)

| Section | Description |
|---|---|
| Custom Report | Use this window to create a report for each data file. |
| System Suitability | Use this window to enter system suitability parameters for each named peak and to perform noise and drift tests for the discrete and multi-chromatogram wavelengths in your method. |
| Properties | This dialog box contains the following pages: Description, options, Calibration, Audit Trail.<br><br>Use this dialog box to do the following:<br><br>• Enter a description of the method<br>• Select the analysis options: Analyze after acquisition (default setting) or Analyze during acquisition<br>• Select the Amount / Area or the Area / Amount response factor definition<br>• Select or clear the automatic averaging of standard replicates<br>• Specify the number of standard replicates in the rolling average<br>• Enable the method audit trail |

# Creating an Acquisition Method

Methods can be created from either the offline Instrument window or the online Instrument window. In this topic, you open the offline Instrument window, and then use the Method Wizard to create a method.

❖ **To create your first method**

1. Opening the Offline Instrument Window
2. Activating the Method Wizard
3. Entering the Instrument Parameters
4. Triggering Data Acquisition
5. Entering the Column Parameters
6. Saving the Method

## Opening the Offline Instrument Window

There is an Online Instrument window and an Offline Instrument window for each instrument in the Enterprise. From the Online Instrument window, you can perform both instrument control and data processing operations. From the Offline Instrument window, you can perform only data processing operations, such as creating methods and reprocessing data files.

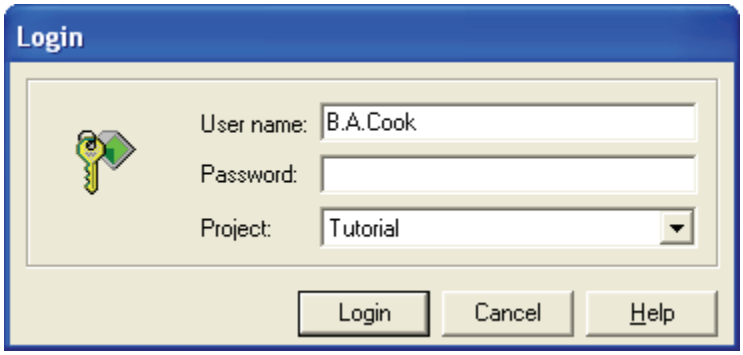
### ❖ To open the Offline Instrument window

1. Do one of the following:
   - For ChromQuest, in the Main Menu window (see Figure 27), right-click the icon that represents your instrument, and then choose **Open Offline** from the shortcut menu. Then go to step 2.
   - For ChromQuest SI, from the computer desktop, choose **Start > All Programs > Chromatography > ChromQuest SI Offline**. Then go to step 4.

   **Note** ChromQuest SI does not contain the Main Menu window and does not provide security or project management features.

2. Depending on which dialog box or window appears, do one of the following:
   - If the Login dialog box shown in Figure 72 appears, the Enable Logins feature has been selected. Continue at step 3.
   - If the Instrument Wizard dialog box appears, as shown in Figure 73, continue at step 5.
   - If the Instrument window is activated as indicated by its title bar, you have successfully opened the Instrument window and are ready to go to the next topic, "Activating the Method Wizard" on page 84.

**Figure 72.** Login dialog box




3. Log in:
   a. Type your user name in the User name box
   b. Type your password in the Password box

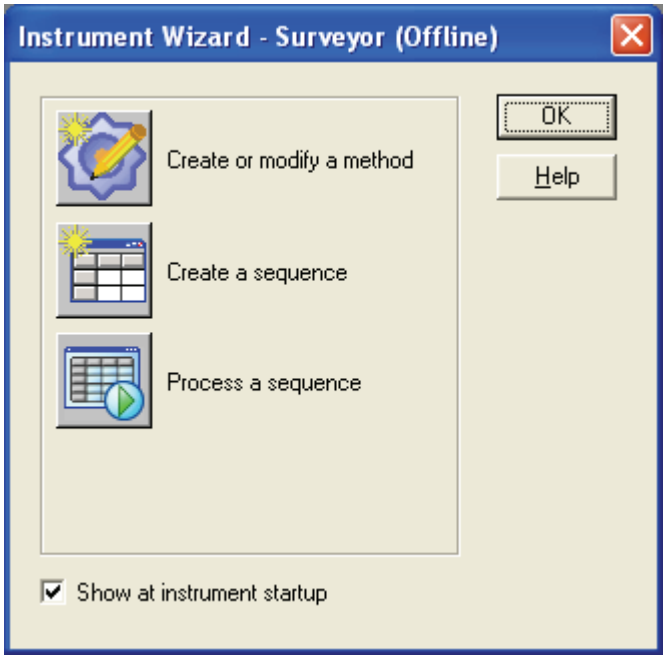
c. Select a project from the Project list. If you created the project, *Tutorial*, as you performed the Creating a Project tutorial beginning on page 47, select it from your project list.

d. Click **Login**.

4. Depending on whether the Instrument window appears, do one of the following:

   - If the Instrument Wizard dialog box appears (see Figure 73), continue at step 5.
   - If the Instrument window is activated as indicated by its title bar, you have successfully opened the Instrument window and are ready to go to the next topic, "Activating the Method Wizard" on page 84.

**Figure 73.** Offline Instrument Wizard dialog box




5. Clear the **Show at instrument startup** check box at the bottom of the Instrument Wizard dialog box.

   You do not use the Instrument Wizard dialog box in this tutorial.

6. Close the Instrument Wizard dialog box to activate the Instrument window.

# Activating the Method Wizard

Before you can inject a sample, you must create a method that contains your instrument control parameters. In this tutorial, you use the Method Wizard to create a new method.

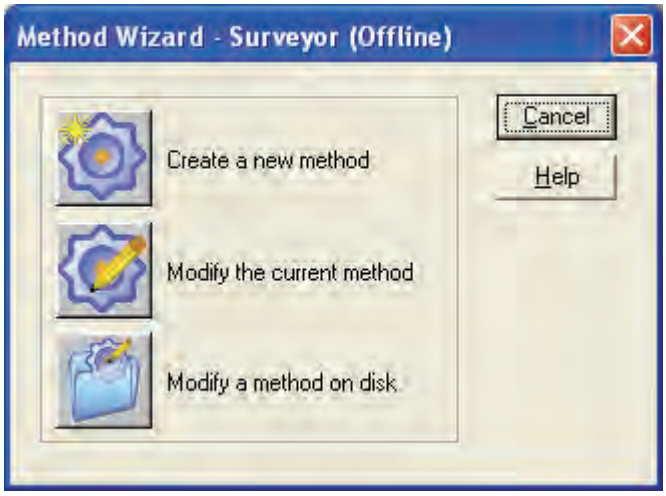
The Method Wizard opens a method that contains default control parameters for each module of the instrument. The Method Wizard also sets up a navigation bar at the bottom of the Instrument window. Clicking the blue arrows in the navigation bar opens the dialog boxes of the method one-by-one.

### ❖ To activate the Method Wizard

1. From the menu bar in the Instrument window, choose **File > Method > Method Wizard**.

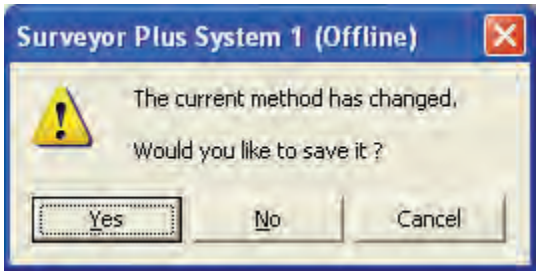
   The Method Wizard (see Figure 74) appears.

   **Figure 74.** Method Wizard dialog box




2. Click the **Create a new method** button.
3. If the dialog box shown in Figure 75 appears, click **No**.

   **Figure 75.** Dialog box that appears when you attempt to open a New Method




You save your method after you enter your instrument setup parameters and your column information.

The Instrument Setup window appears. The Method dialog buttons appear at the bottom of the Instrument window. The active method is the default method named “untitled” and the instrument setup parameters are those contained in the default method.

## Entering the Instrument Parameters

The Instrument Setup window contains a page for each configured module. Enter the chromatographic conditions of your method by performing the following procedures that apply to your Surveyor Plus system:

- Entering the Pump Parameters
- Entering the Autosampler Parameters
- Entering the PDA Detector Parameters
- Entering the UV/Vis Detector Parameters
- Entering the FL Detector Parameters
- Entering the RI Detector Parameters

### Entering the Pump Parameters

The Surveyor LC Pump Plus is a quaternary gradient pump capable of proportioning four solvents and performing gradient programs containing up to 40 lines.

In this tutorial, you run the pump in the isocratic mode. This means that the mobile phase composition remains constant during the run. You program the pump to create a binary mobile phase consisting of 80% methanol and 20% water (v/v). This saves you the time and effort of preparing a pre-mixed mobile phase.

In addition to entering the parameters to create a binary mobile phase, you enter the conditions under which the pump will continue to operate. You use the minimum and maximum pressure boxes, shown in Figure 76, to program the pump to stop if the backpressure falls below or rises above an unacceptable level.

A drop in the system backpressure is typically caused by a leak in the solvent lines. A rise in the system backpressure is typically caused by column degradation or a clogged frit in the line body at the top of the purge manifold (see Figure 5) of the pump.

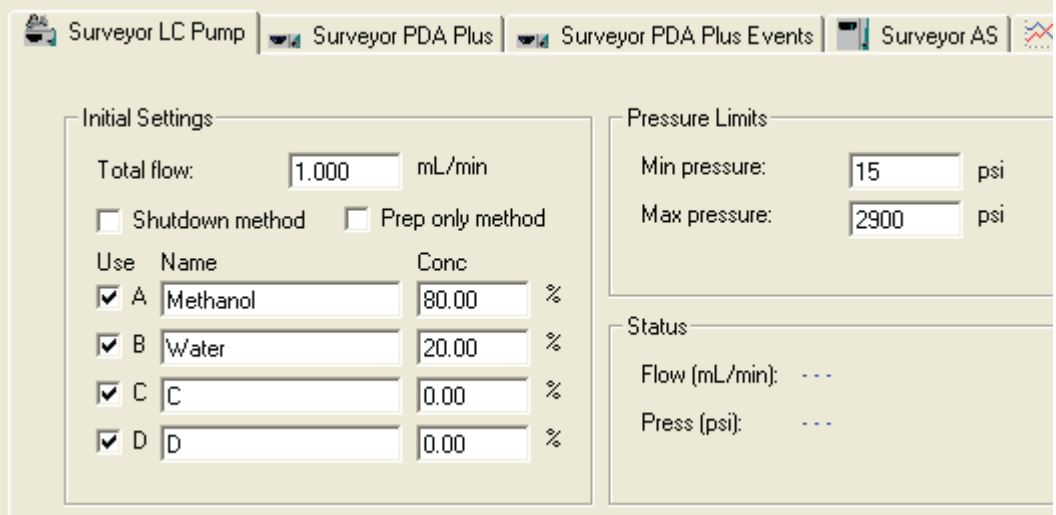
### ❖ To enter the parameters for the Surveyor LC Pump Plus

1. In the Instrument Setup window, click the **Surveyor LC Pump** tab to open the Surveyor LC Pump page. See Figure 76.
2. Keep all parameters in the Surveyor LC Pump page set to the default settings except those that are shown in the following table and in Figure 76.

   After you enter a value of 80 in the Conc box for solvent line A, ChromQuest automatically adjusts the concentration of solvent line B to 20.

| Parameter | Setting | Result |
|---|---|---|
| Initial Settings | | |
| • Methanol | Specifies Methanol as the name for solvent A. The solvent name appears in the Method report. | |
| | Conc: 80 | Specifies that the initial composition of the mobile phase is 80% Methanol by volume. |
| • Water | Specifies Water as the name for solvent B. The solvent name appears in the Method report. | |
| | Con: 20 | Specifies that initially the mobile phase will consist of 20% water by volume. |
| Pressure Limits | | |
| • Min Pressure | 15 (psi) or 1 (bar) or 0.1 (MPa) | Specifies the minimum system backpressure at which the pump will continue to run. If the backpressure falls below this limit, which happens when a leak occurs, the pump pistons will stop. |
| • Max Pressure | 2900 (psi)<br>(20 MPa = 200 bar = 2900 psi) | Specifies the maximum backpressure at which the pump will continue to run. |

**Figure 76.** Surveyor LC Pump page with parameters for the pump




## Entering the Autosampler Parameters

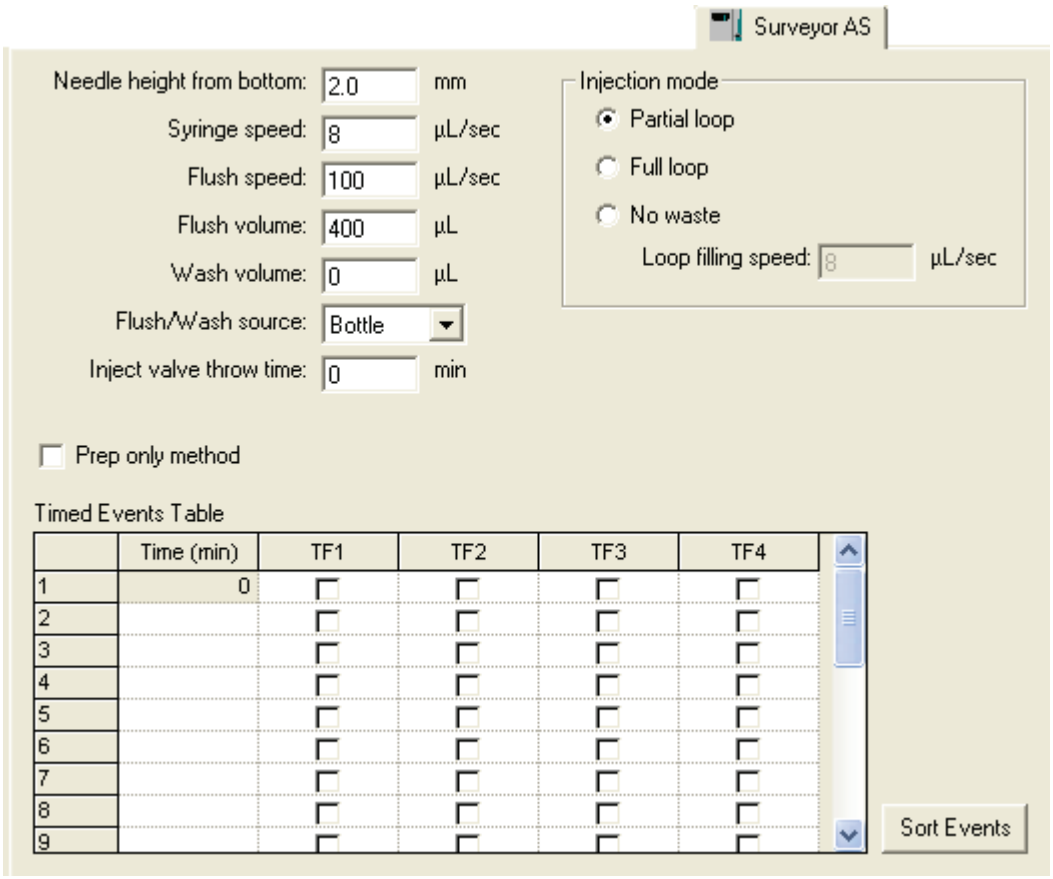
In this tutorial, you use the partial loop injection mode because you want to be able to inject variable amounts of sample.

### ❖ To enter the parameters for the Surveyor Autosampler Plus

1. Click the **Surveyor AS** tab to open the Instrument Setup page for the Surveyor Autosampler.
2. Use the default settings shown in Figure 77.

> **Note** If you are using water or a water / methanol mixture for the flush solvent, lower the flush speed to less than 100 µL/sec. If you hear the syringe drive rattle, the flush speed is too high.

**Figure 77.** Surveyor AS page




## Entering the PDA Detector Parameters

The Surveyor PDA Plus Detector is a photodiode array detector that is capable of scanning the UV/Vis range from 190 to 800 nm. In addition to collecting scan data, the detector is capable of simultaneously collecting up to three discrete channels. You can use this capability to set the bandwidth and data rate parameters for the discrete channels separately from those of the scan data.

In this tutorial, you program the PDA detector to collect scan data from 220 to 300 nm at a step size of 1 nm. In addition, you program the detector to collect two discrete channels.

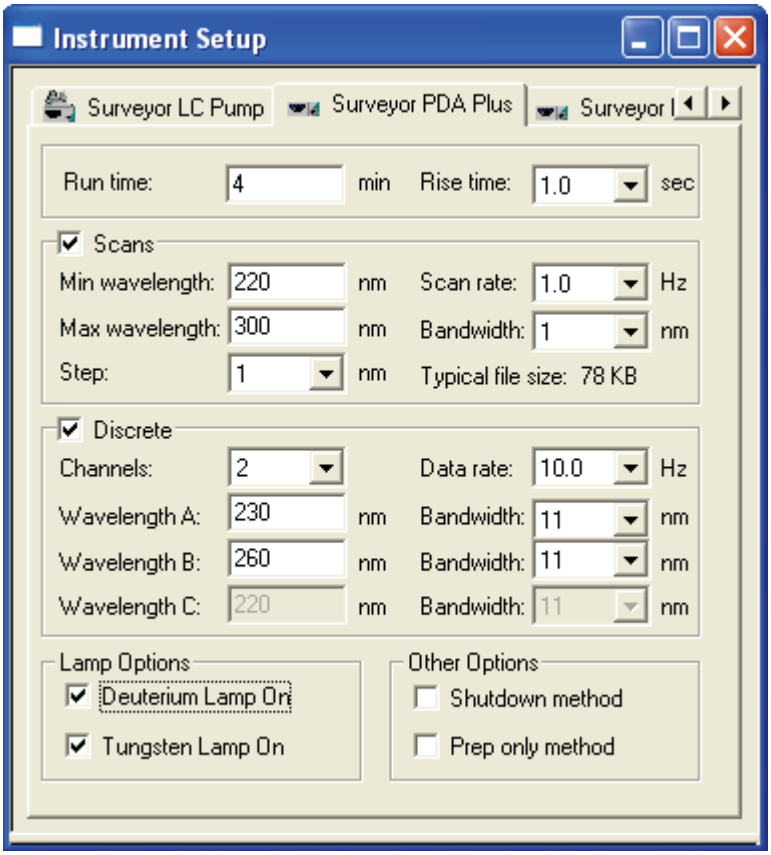
**Note** Because the spectral analysis program compares spectra on a wavelength to wavelength basis, Set the step size to 1 nm when collecting scan data to create spectrum files. If you collect one spectrum from 200 to 300 nm with a step size of 2 nm, and another spectrum from 201 to 301 nm with a step size of 2 nm; these spectra will have no wavelengths in common. Therefore, even if the spectra are of the same analyte, the comparison algorithm will report a match of 0.

### ❖ To enter the parameters for the Surveyor PDA Plus Detector

1. Click the **Surveyor PDA Plus** tab to open the detector page.
2. Keep all parameters in the Surveyor PDA Plus dialog box set to the default settings except those that are shown in the following table and in Figure 78.

| Parameter | Setting | Result |
|---|---|---|
| Run time | 4 | Acquires data for 4 minutes |
| Rise time | 1 | Specifies a rise time of 1 second |
| Scans | | |
| • Step | 1 | Collects a chromatogram for every wavelength in the scan range |
| • Bandwidth | 1 | Specifies a spectral resolution of 1 nm |
| Discrete | | |
| • Channels | 2 | Collects data for two discrete wavelengths |
| • Wavelength A | 230 | Collects absorbance data for 230 nm |
| • Wavelength B | 260 | Collects absorbance data for 260 nm |

**Figure 78.** Surveyor PDA Plus page



## Entering the UV/Vis Detector Parameters

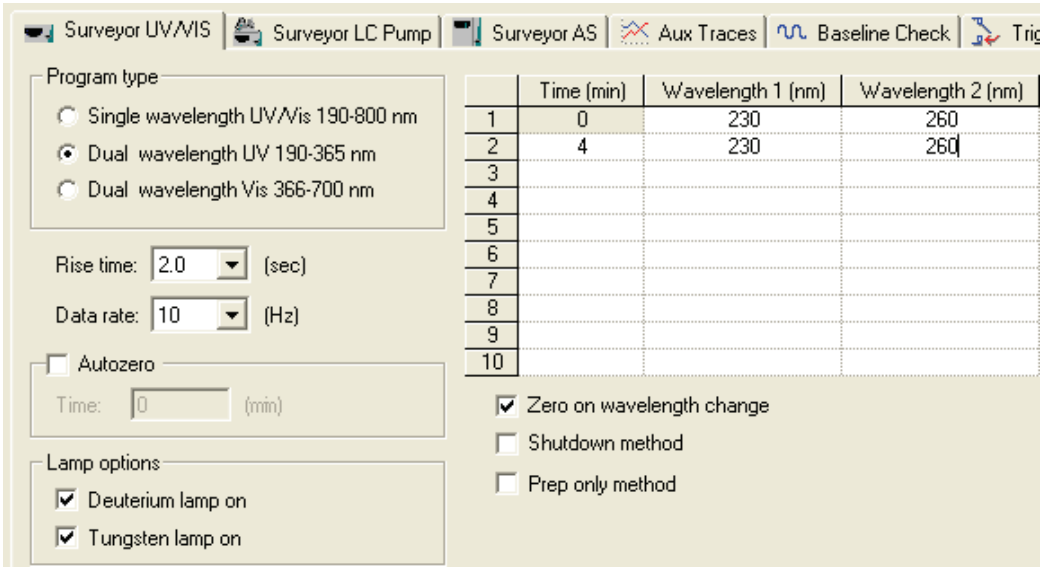
The Surveyor UV/Vis Plus Detector is a programmable, dual-wavelength detector. In this tutorial, you run the detector in the dual-wavelength mode and collect absorbance data for a period of 4 minutes. At the end of your first injection, you will have a data file containing two chromatograms (230 nm and 260 nm).

### ❖ To enter the parameters for the Surveyor UV/Vis Plus Detector

1. Click the **Surveyor UV/Vis** tab to open the detector page.
2. Keep all parameters in the Surveyor UV/Vis dialog box set to the default settings except those that are shown in the following table and in Figure 79.

| Parameter | Setting | Result |
|---|---|---|
| Program Type | | |
| • Program Type | Dual wavelength 190 to 365 nm | Specifies that two chromatograms in the UV/Vis range will be collected |
| Wavelength Table | | |
| • Row 1 | Time = 0,<br>Wavelength 1 = 230 nm,<br>Wavelength 2 = 260 nm | Specifies that the wavelength program will collect data at 230 nm and 260 nm for a period of 4 minutes |
| • Row 2 | Time = 4,<br>Wavelength 1 = 230 nm,<br>Wavelength 2 = 260 nm | |

**Figure 79.** Surveyor UV/Vis page



## Entering the FL Detector Parameters

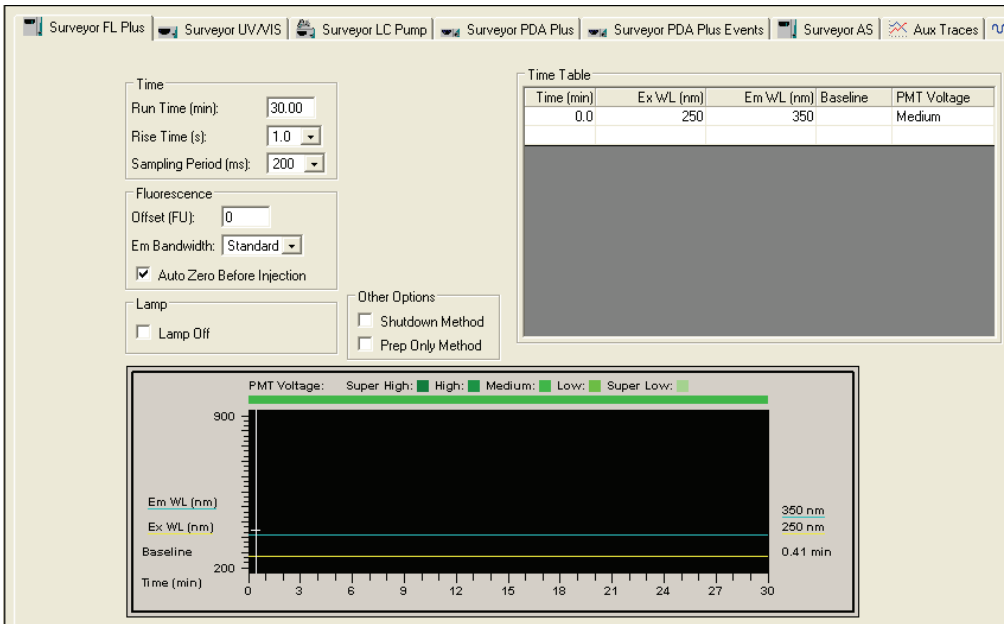
To perform the tutorial, enter the chromatographic conditions for the analyte that you plan to inject.

### ❖ To specify the parameters for the FL detector

1. Click the **Surveyor FL Plus** tab to open the method page for the FL detector.

   Figure 80 shows the default parameters for the Surveyor FL Plus Detector.

   **Figure 80.** Surveyor FL Plus page




2. Enter the FL detector settings for your application as follows:

   a. In the Run Time box, type the length of time that you want the detector to acquire data.

      Because the program cannot collect more than 72000 data points per run, the allowable run time depends on the sampling period. For a sampling period of 50 ms (20 Hz), the maximum allowable run time is 60 minutes. For a sampling period of 800 ms (1.25 Hz), the maximum allowable run time is 600 minutes.

   b. In the Rise Time list, select a rise time that is appropriate for your application.

      Increasing the rise time filter decreases the baseline noise, but it can also result in peak broadening. In general, select a rise time that is no higher than one-tenth the base peak width for the narrowest peak of interest.

   c. In the Sampling Period list, select a sampling period based the on the run time of your analysis and the expected baseline peak width of the narrowest peak in your chromatogram.

As you increase the run time beyond 60 minutes, you must increase the sampling period above the minimum sampling period of 50 ms (20 Hz) because the program cannot collect more than 72000 data points per run.

To integrate a peak, you must collect a minimum of three points across the peak. For optimal integration, collect at least 20 points across the peak.

d. In the Offset box, keep the value at the default of **0** for most applications.

e. In the Em bandwidth list, select **Standard** (15 nm) or **Wide** (30nm).

f. If necessary, select the **Autozero Before Injection** check box to compensate for baseline drift during a sequence run.

3. In the Time Table, enter the initial conditions or the conditions for the duration of the run:

   a. In the Ex WL column, type an excitation wavelength from 200 nm to 800 nm, and then press ENTER.

   b. In the Em WL column, type an emission wavelength from 250 nm to 900 nm, and then press ENTER.

   c. In the PMT Voltage column, select a PMT voltage.

      Increasing the PMT voltage increases the sensitivity of the analysis, but also shortens the operating lifetime of the photomultiplier.

      If the program contains one time line, the specified conditions remain the same for the time period specified in the Run Time box.

4. To change the excitation wavelength, emission wavelength, or PMT Voltage settings or to zero the baseline during the run, enter more time lines in the time table:

   a. In the Time column, type a time value from 0.1 to 600.0 minutes for the second row onward.

   b. Type appropriate excitation and emission wavelengths in their respective columns.

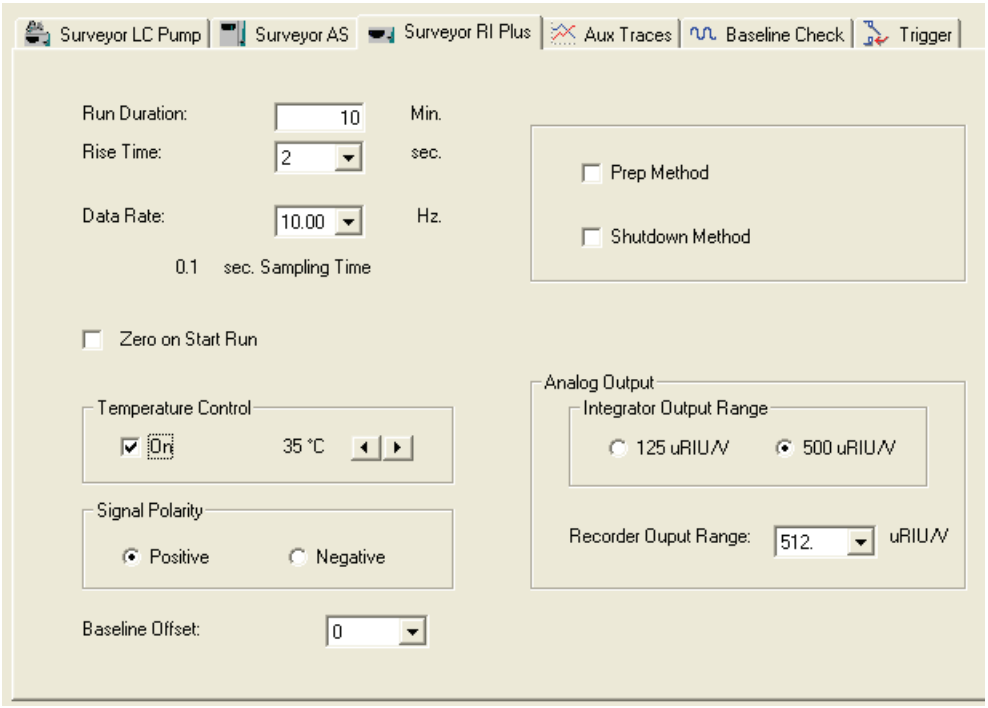
   c. In the Baseline column, select **Autozero** to zero the baseline at the specified time point or Hold to leave the baseline unchanged.

## Entering the RI Detector Parameters

### ❖ To specify the parameters for the RI detector

1. Click the **Surveyor RI Plus** tab to open the Surveyor RI Plus method parameters page, shown in Figure 81.

   **Figure 81.** Surveyor RI Plus Detector method parameters page




2. Enter the instrument control parameters for the RI detector as follows:

   a. In the Run Duration box, type a value to specify the length of time in minutes that data is to be acquired from the detector during the run.

   b. From the Rise Time list, select a value to specify the response time of the detector in seconds.

      Increasing the rise time reduces the baseline noise. However, setting the rise time to a value greater than one-tenth the width of the chromatographic peak at half-height results in peak broadening. The 2 second default value is appropriate for most LC applications.

   c. From the Data Rate list, select a value.

      For optimal results, select a data rate that collects at least 20 points across the peaks of interest.

   d. Select the **Zero on Start Run** check box to zero the chromatographic baseline at the start of a run.

      You can use this feature to compensate for baseline drift during a sequence run.

e. Select the **Temperature Control** check box to turn on the temperature control feature.

   Because RI measurements are very sensitive to temperature, use this feature to improve the reliability of your analyses.

f. Select the signal polarity option for your analysis that produces chromatograms with positive peaks, rather than negative dips.

   If the analyte has a higher refractive index than the mobile phase, click the **Positive** option. If the analyte has a lower refractive index than the mobile phase, click the **Negative** option.

   The Baseline Offset, Integrator Output Range, and Recorder Output Range parameters are used to scale the output to an integrator or a recorder.

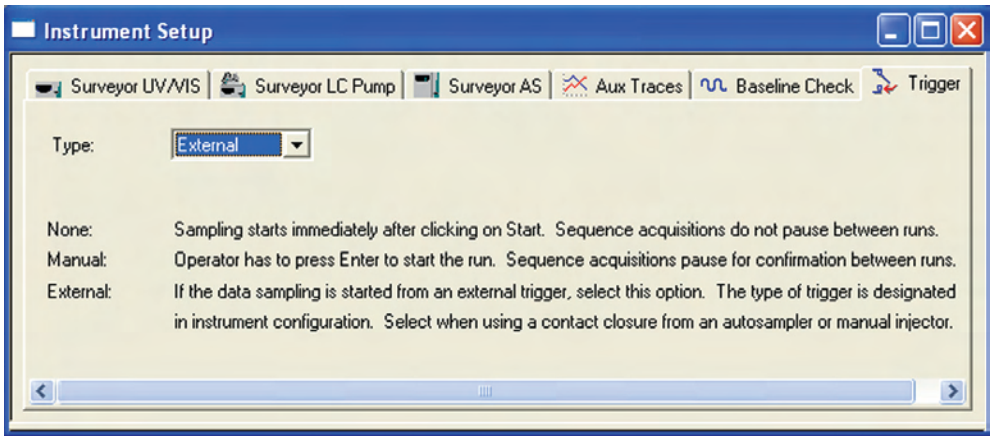
## Triggering Data Acquisition

### ❖ To specify the trigger type for your method

1. From the Instrument Setup window, click the **Trigger** tab to open the Trigger page.
2. Select a trigger type in the Type list.

   If your instrument contains a Surveyor Autosampler Plus, use the **External** trigger mode to start your runs. See Figure 82.

**Figure 82.** Trigger page

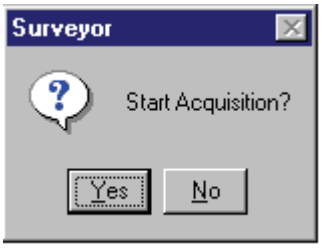



When you select External as the triggering mode, the autosampler triggers the detector to begin data acquisition.

**Note** If your run remains in the Waiting For Trigger status mode, check the system interconnect cable connections on the back of your instrument and verify that the autosampler is configured properly.

If your instrument does not contain an autosampler, you might choose to Trigger data acquisition by selecting **None** or **Manual** from the Type list. If you select **Manual**, the Start Acquisition dialog box pops up between each run in a sequence table. See Figure 83.

**Figure 83.** Start Acquisition dialog box




## Entering the Column Parameters

Adding column parameters to the method enables ChromQuest to calculate performance parameters for your chromatographic separation. You can add the column parameters to your method either before or after data acquisition.

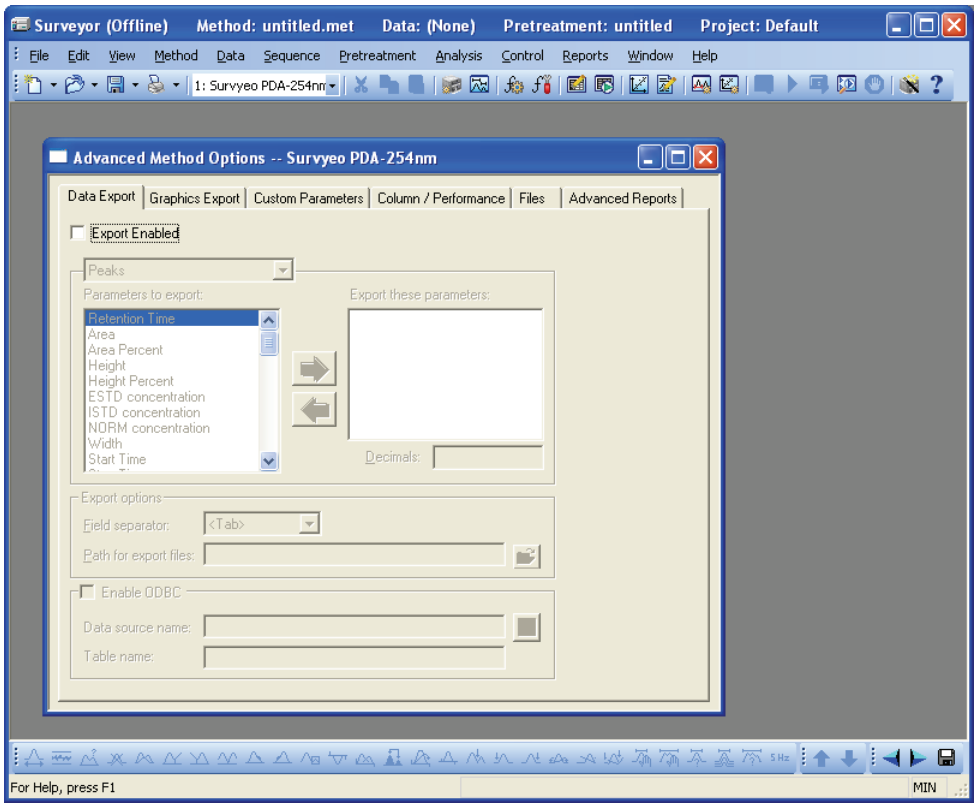
**Note** ChromQuest calculates performance parameters on a per detector basis.

### ❖ To enter the parameters for your column to the method

1. Click the right blue navigation arrow at the bottom left of the Instrument window. Continue to click the arrow until the Advanced Method Options window appears.

   On the first click, the blue arrow opens the Integration Events dialog box. On the second click, the blue arrow opens the Peak / Group Tables dialog box. On the third click, the blue arrow opens the Advanced Method Options window. See Figure 84.

**Figure 84.** Advanced Method Options window




2. In the Advanced Method Options window, click the **Column / Performance** tab to open the Column / Performance page.

3. Enter your column information. If you are using a Hypersil C-18, 4.6 × 100 mm, 5 micron particle size column, make the entries that are listed in the following table and shown in Figure 85.

4. Select the **Calculate performance parameters for this channel** check box.

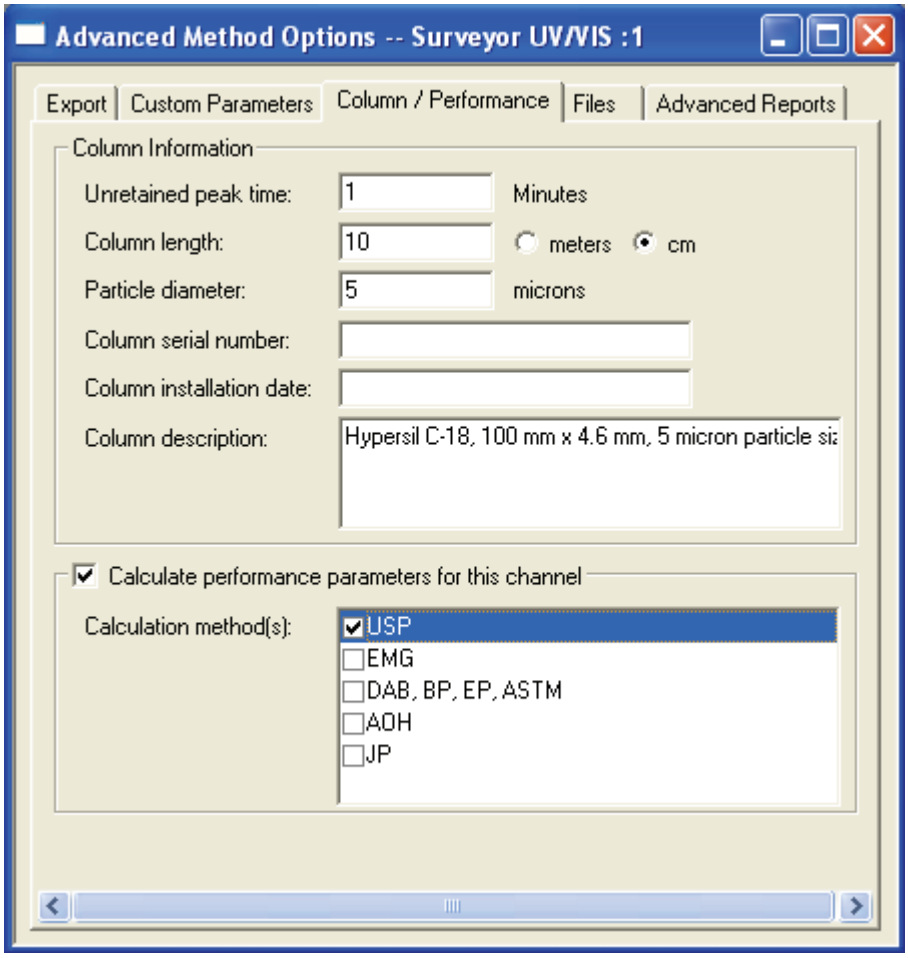
   ChromQuest calculates performance parameters on a per detector basis. Selecting the **Calculate performance parameters for this channel** check box enables the calculation of performance parameters for all the chromatograms produced by the detector listed in the Channel Selector list and the title bar of the window.

5. If your instrument contains two detectors (for example, a UV/Vis detector and an RI detector) and you want to report performance parameters for the second detector as well, select the second detector in the Channel Selector list, and then select the **Calculate performance parameters for this channel** check box a second time.

| Parameter | Setting | Result |
|---|---|---|
| Column Information | | |
| • Unretained Peak Time | 1 | Specifies the void volume of the LC column in min [volume (mL) × flow rate (mL/min)] |
| • Column Length* | cm | Specifies the units of length as centimeters |
| • Column Length | 10 | Specifies a length of 10 cm |
| • Particle Diameter | 5 | Specifies that the column packing material has an average diameter of 5 microns |
| • Column Description | Descriptive information about your column | The information provided in this box can be printed in the method report. |
| Calculate Performance Parameters For This Channel | | |
| • Calculate Performance Parameters For This Channel | ☑ | Specifies that performance parameters will be calculated for the detector listed in the title bar of the window |
| • Calculation Methods | ☑ USP | Specifies that the performance parameters will be calculated using the USP equations |

*Note. Select the centimeters option, and then type the length of your column in the column length box.

**Figure 85.** Column/Performance page of the Advanced Method Options window




ChromQuest requires your column information and a calculation method to calculate parameters such as the capacity factor, asymmetry, and resolution for a chromatographic peak. If you attempt to add annotations for these parameters to your chromatograms before you add your column information to the method, ChromQuest reports these values as zero.

## Saving the Method

### ❖ To save your new method

1. Click the **Save Method As** button at the bottom of the Instrument window.

   The Save Method As dialog box, shown in Figure 86, appears.

2. In the Save Method As dialog box, browse to the appropriate directory for your method file if you are not logged into a project.

   If you are logged into the Tutorial project, the method is saved in the following folder:

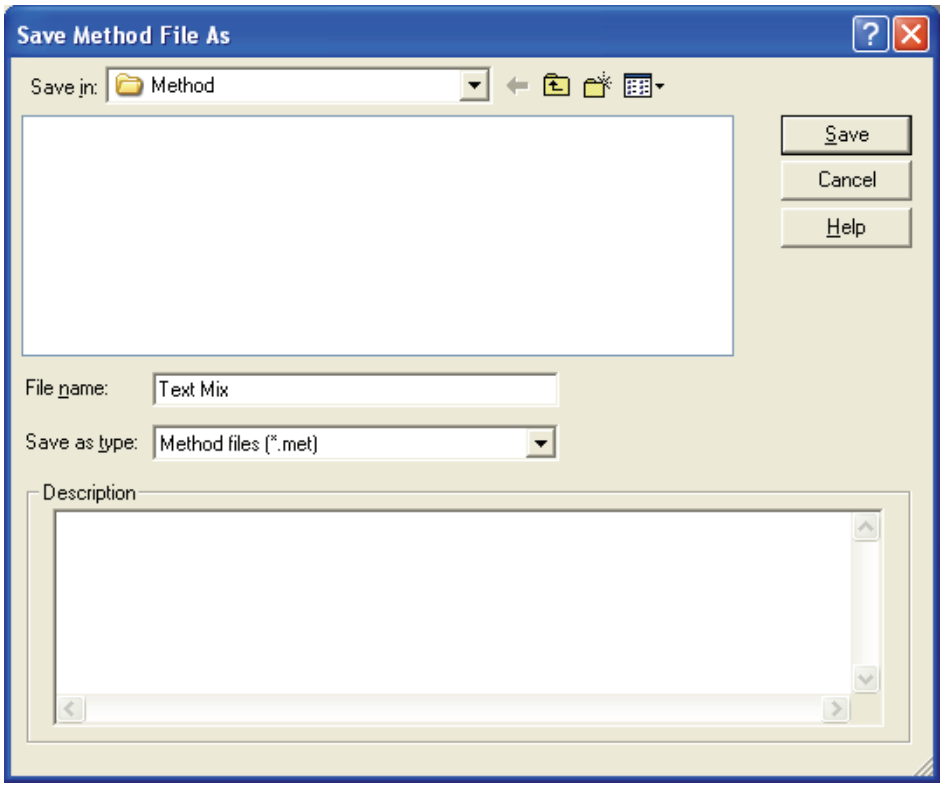
   Drive:\ChromQuest\Projects\Tutorial\Methods.

3. Type the name **Test Mix** in the File Name box, and then click **Save**.

   ChromQuest adds the .met file extension to method files.

4. Do not exit the Instrument Setup window. Proceed to the next topic, **Creating a Shutdown Method**, which describes how to create a shutdown method by modifying an acquisition method.

**Figure 86.** Save Method File As dialog box



# Creating a Shutdown Method

To automatically turn off the pump flow, the detector lamps, or both at the end of a sequence run, the last line of the sequence table must contain a shutdown method. You cannot use a shutdown method to make injections. Creating sequence tables is discussed in Chapter 10, "Creating a Sequence Table."

For a shutdown method to be valid, the Shutdown check boxes in the method pages for the pump and the detectors must be selected, even if you do not want to turn off the lamps or the solvent flow.

### ❖ To create a shutdown method

1. If the Instrument Setup window is not open, open it by choosing **Method > Instrument Setup** from the Instrument window menu bar.
2. If your method, Test Mix.met, is not open, open it:
   a. From the Instrument window menu bar, choose **File > Method > Open.**

      The Open Method File dialog box appears.
   b. Browse to the appropriate directory, and then select the Test Mix.met method that you created while performing this tutorial.
3. To set the shutdown parameters for the detector:
   a. In the Instrument Setup window, click the tab for your detector to open its method parameters page.
   b. Select the **Shutdown Method** check box.
   c. To turn off the lamp(s) at the end of a sequence run, do the following:
      - For the Surveyor UV/Vis Plus Detector and the Surveyor PDA Plus Detector, clear the check boxes for the lamps.
      - For the Surveyor FL Plus Detector, select the Lamp Off check box.

      **Note** For the Surveyor RI Plus Detector, you can only turn off the lamp by turning off the power to the detector. The operating lifetime of the lamp is 4.5 years.
   d. Verify that you have entered the appropriate parameter settings:
      - Figure 87 shows the settings for the Surveyor UV/Vis Detector.
      - Figure 88 shows the settings for the Surveyor PDA Plus Detector.

**Figure 87.** Instrument setup page for the Surveyor UV/Vis Plus Detector (showing the parameters for a shutdown method)

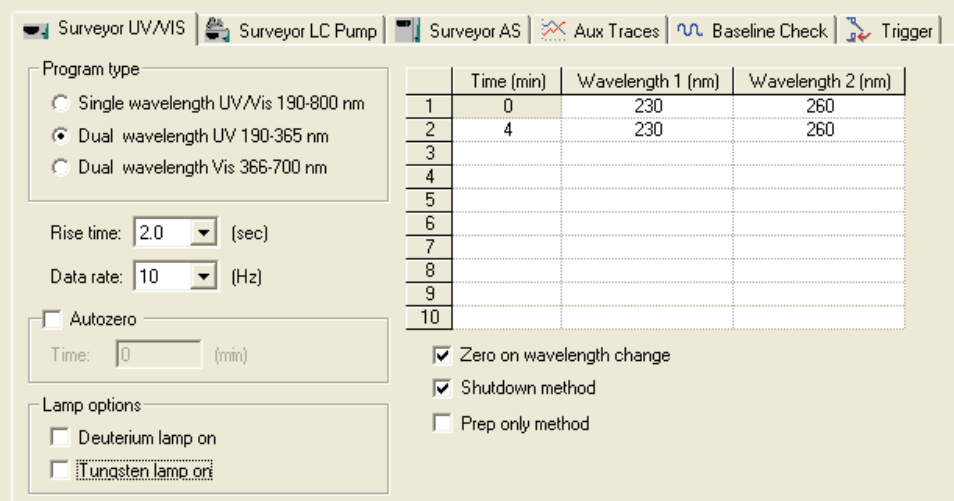
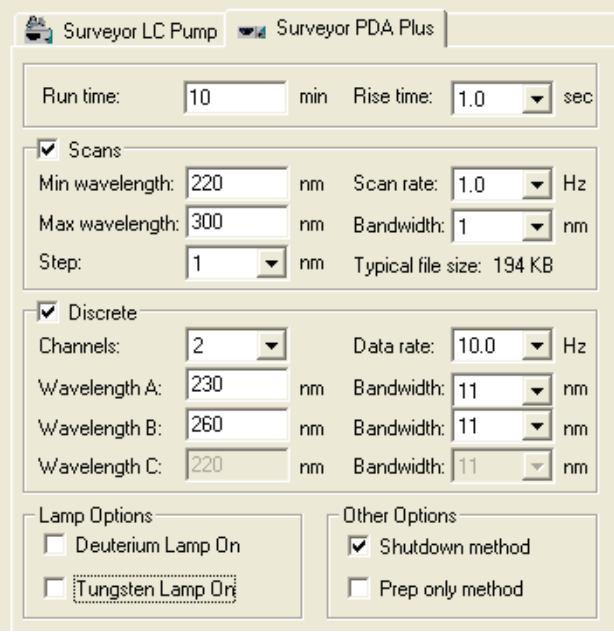


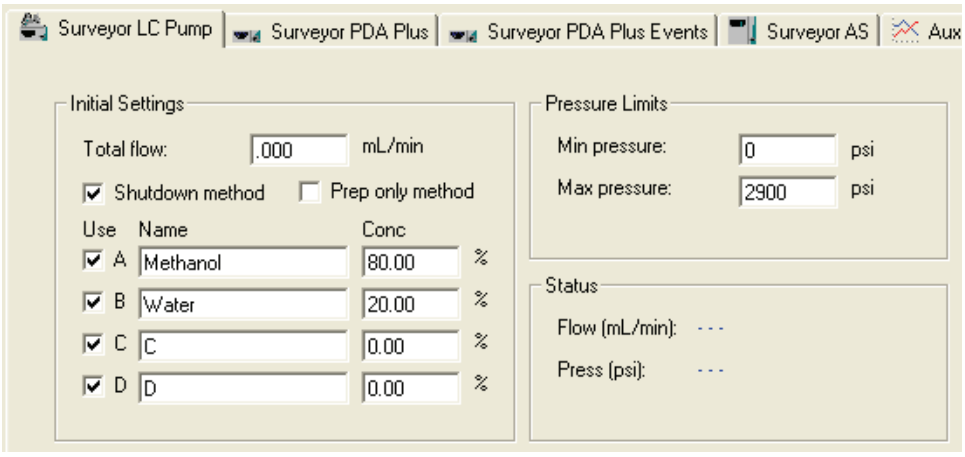

**Figure 88.** Instrument Setup window for the Surveyor PDA Plus Detector (showing the parameters for a shutdown method)




4. To turn off the pump flow at the end of a sequence run:
   a. Click the **Surveyor LC Pump** tab to open the Surveyor LC Pump page.
   b. Keep all parameters in the pump page the same as the settings for the acquisition method, Test Mix.met, except those that are shown in the following table and in Figure 89.

| Parameter | Setting | Result |
|---|---|---|
| Initial Settings | | |
| • Shutdown Method | ◉ | Specifies that this method is a shutdown method |
| • Total Flow | 0 | Pump flow will be turned Off |
| Pressure Limits | | |
| • Min Pressure | 0 | Allows a minimum backpressure of 0 |

**Figure 89.** Instrument Setup page for the Surveyor LC Pump (showing the parameters for a shutdown method)




5. Save the method as Shutdown.met:
   a. From the Instrument window menu bar, choose **File > Method > Save As** to open the Save Method As dialog box.
   b. Type **Shutdown** in the Filename box.
   c. Click **Save**.

ChromQuest saves methods with the .met file extension.

# Preparing Your Instrument for a Run

In this tutorial, you learn how to open the online Instrument window, monitor the status of your instrument, remove air from the solvent lines, download a method, and check the stability of the baseline.

Before you can perform this tutorial, you must configure your Surveyor Plus system. If your instrument has not been configured, see Chapter 3, "Configuring Your Instrument." Check the configuration for the autosampler, and ensure that the Verify Door Is Closed option is not selected.

To perform this tutorial, you must have the following items:

- HPLC-grade water
- HPLC-grade methanol (not required for the RI detector)
- Hypersil, 100 × 4.6 mm, C-18 column, 5 micron particle size or equivalent

To prepare your instrument for a run, perform the procedures that apply to your instrument in the order listed:

1. Setting Up the System
2. Turning On the Power
3. Opening the Online Instrument Window
4. Checking the Status of the Instrument Modules
5. Removing Air from the Solvent Lines
6. Purging the Flow Cell of the RI Detector
7. Downloading the Method
8. Checking the Stability of the Baseline

# Setting Up the System

The following instructions describe how to set up the Surveyor LC system so that you can perform the remaining tutorials contained in this manual. These tutorials guide you through the process of injecting a sample and reporting the results of your run.

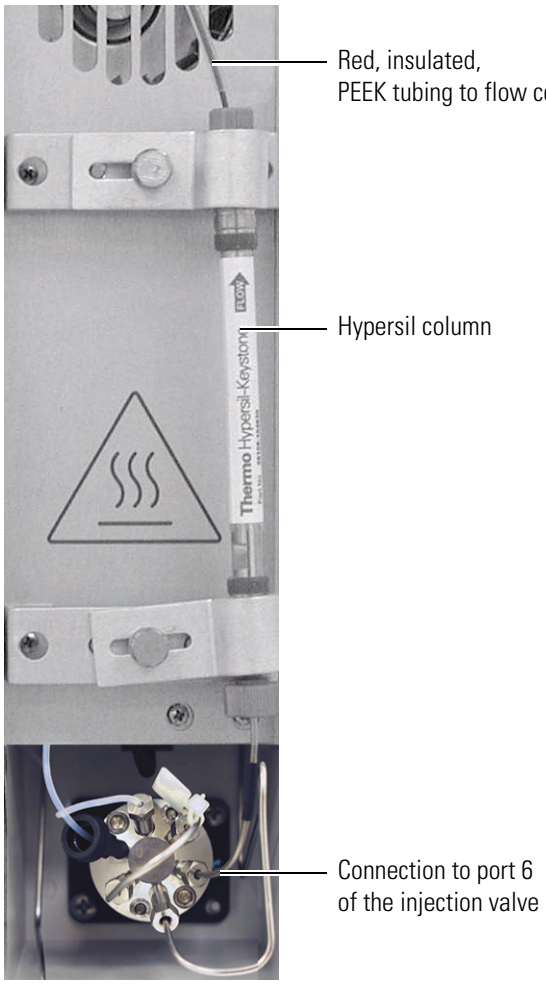
### ❖ To set up the LC system

1. Fill the wash bottle with 90:10 methanol / water (v/v) or 100% methanol or an appropriate solvent for your chromatographic method.
2. Fill the solvent reservoir bottle A with HPLC-grade methanol or an appropriate solvent for your chromatographic method.
3. Fill the solvent reservoir bottle B with HPLC-grade water or an appropriate solvent for your chromatographic method.
4. Install a standard sample tray into the tray compartment of the autosampler.

   The Surveyor Autosampler is shipped with four standard sample trays in the tray compartment. The standard sample tray holds forty, 1.8 mL standard vials.
5. Connect the LC Column (see Figure 90):
   a. If it has not already been done, connect high-pressure tubing to port 6 of the Valco injection valve. Use a high-pressure fitting to connect the other end of the tubing to the inlet of the LC Column.
   b. Use a high-pressure fitting to connect the outlet of the LC column to the tubing that is attached to the flow cell of the detector.

**Note** If you are using a LightPipe flowcell, make sure that you are also using the insulated, red, PEEK tubing that is supplied with the LightPipe. This insulated tubing minimizes temperature fluctuations.

**Figure 90.** Valco injection valve and column connections




# Turning On the Power

Before you launch ChromQuest from the Windows desktop, turn on the power to each module of your instrument. The power switch for each module is located below its left door, as shown in Figure 91.

Shortly after you switch on the power, the Power LEDs and the Run LEDs turn green and the autosampler syringe performs its initialization process of homing the syringe. The homing process takes approximately 20 seconds. You can hear the movement of the syringe drive as the plungers are homed.

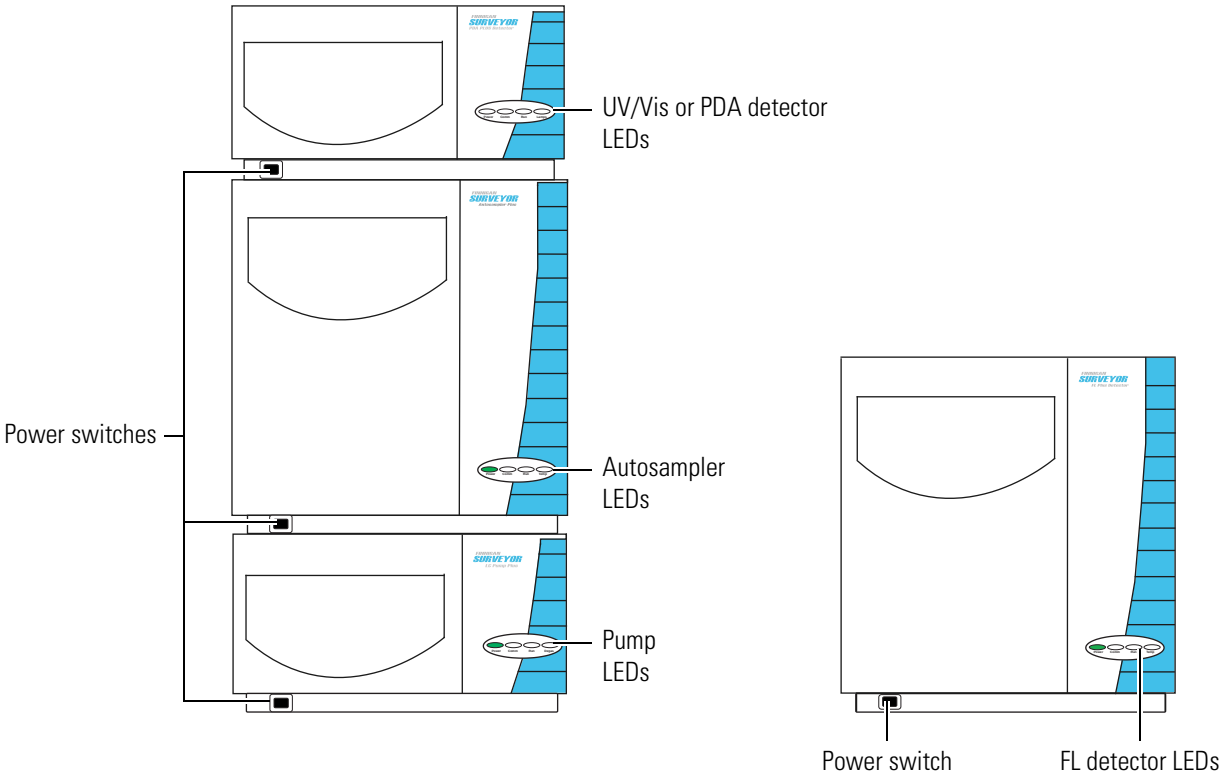
If the temperature control feature for the RI detector is On, the Temp and Run LEDs for the detector do not turn green until the operating temperature of the detector stabilizes to the set temperature.

As you power up your instrument, you might encounter the following problems:

- The Power LEDs remain amber. If this happens, make sure that the power line to the affected module(s) is firmly connected.
- The Degas LED on the pump flashes amber. If this happens, the degas unit has failed to produce a vacuum, and you need to call your local Thermo Fisher Scientific representative for repairs.
- The Lamp LED remains amber. If this happens, most likely the lamps are turned off. Continue on with this tutorial. You will turn on the lamps of the Surveyor PDA Plus Detector in the topic, "Checking the Status of the Instrument Modules" on page 110. You will turn on the lamps of the Surveyor UV/Vis Plus Detector in the topic, "Downloading the Method" on page 119.

If your instrument modules powered up correctly, go to the next topic, "Opening the Online Instrument Window" on page 107.

**Figure 91.** Surveyor stack, showing power switches and LEDs



# Opening the Online Instrument Window

There is an online Instrument window and an offline Instrument window for each instrument in The Enterprise. From the online Instrument window, you can perform both instrument control and data processing operations. In this tutorial you learn how to use some of the instrument control features of ChromQuest available in the online Instrument window.

Before you open the online Instrument window, the Comm LEDs on the modules of your instrument are amber. After you open the online Instrument window, the Comm LEDs change from amber to green. In addition, the autosampler syringe goes through its initialization process.

**Note** Before you open the Online Instrument window, wait for the Comm LEDs to illuminate green and for the syringe to complete its initialization process.

After you turn on the power to the modules of your instrument, start ChromQuest and open the online Instrument window.

### ❖ To open the online Instrument window from the computer desktop

1. Do one of the following:
   - To start ChromQuest, go to step 2.
   - To start ChromQuest SI, choose **Start > All Programs > Chromatography > ChromQuest SI**. The Instrument window appears. Go to step 6.
2. Start ChromQuest by choosing **Start > All Programs > Chromatography > ChromQuest** or by double-clicking the ChromQuest application icon.

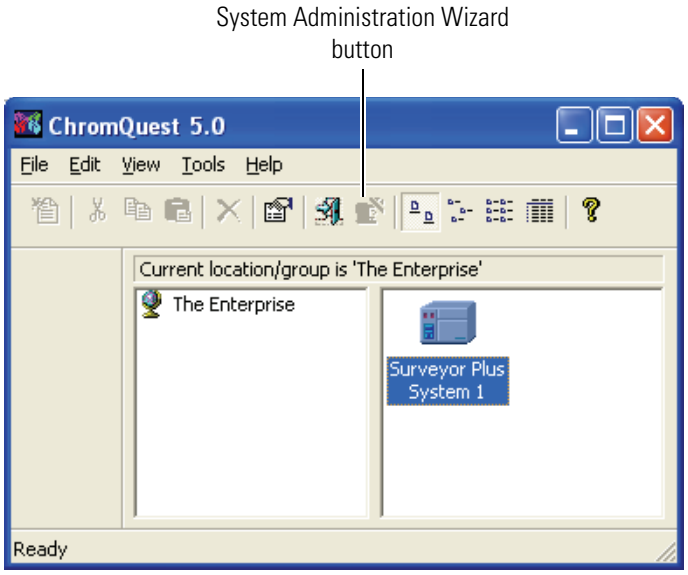
   The Main Menu window of ChromQuest appears. See Figure 92. Instrument and System Administration are performed from this window.


Locked


Unlocked

In the Main Menu window toolbar, shown in Figure 92, notice that the System Administration Wizard button is grayed out (Locked). This means that the Enable Login and Project Management feature is enabled. To open an instrument or perform any administrative activities, you must log in.

**Figure 92.** Main Menu window



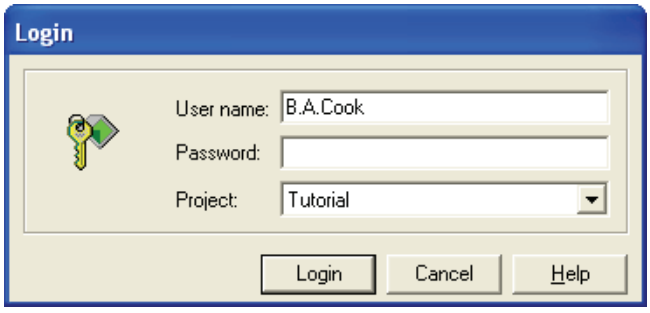

Also notice that the right panel of the window in Figure 92 contains one instrument: Surveyor Plus System 1. The Main Menu window displayed on your computer screen contains the Enterprise that you or your System Administrator created. If the right panel of the Main Menu window does not contain any instruments, see Chapter 3, "Configuring Your Instrument," which contains instructions on how to add an instrument to The Enterprise. In addition, Chapter 3 contains instructions on how to configure your instrument for the tutorials contained in this manual.

3. Right-click the icon that represents your instrument, and then choose **Open** from the shortcut menu.

4. Depending on which dialog box or window appears, do one of the following:

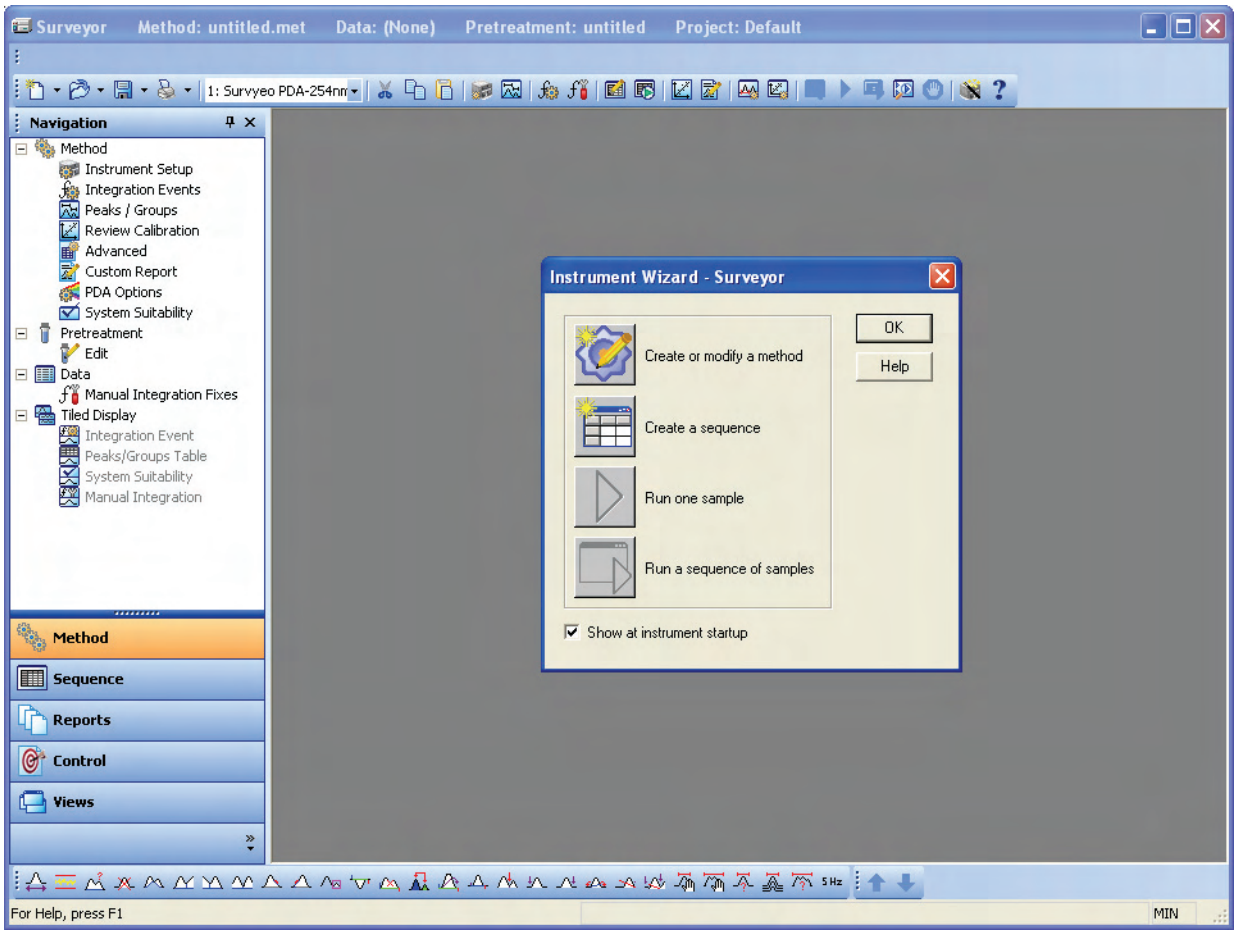
   - If the Login dialog box appears, go to step 5. See Figure 93.
   - If the Instrument Wizard appears, go to step 6. See Figure 94.
   - If the Instrument window is activated as indicated by its title bar, you have successfully opened the Instrument window and are ready to go to the next topic, "Checking the Status of the Instrument Modules" on page 110.

**Figure 93.** Login dialog box



5. Log in:
   a. Type your user name in the User Name box.
   b. Type your password in the Password box.
   c. Select a project from the Project list.
   d. Click **Login**:
      - If the Instrument Wizard appears, go to step 3. See Figure 94.
      - If the Instrument window is activated as indicated by its title bar, you have successfully opened the Instrument window and are ready to go "Checking the Status of the Instrument Modules" on page 110.
6. Clear the **Show at Instrument Startup** check box at the bottom of the Instrument Wizard dialog box. You do not use the Instrument Wizard in this tutorial.
7. Close the Instrument Wizard to activate the Instrument window.
8. Close the Navigation bar. You do not use it in this tutorial.

**Figure 94.** Instrument window at startup



# Checking the Status of the Instrument Modules

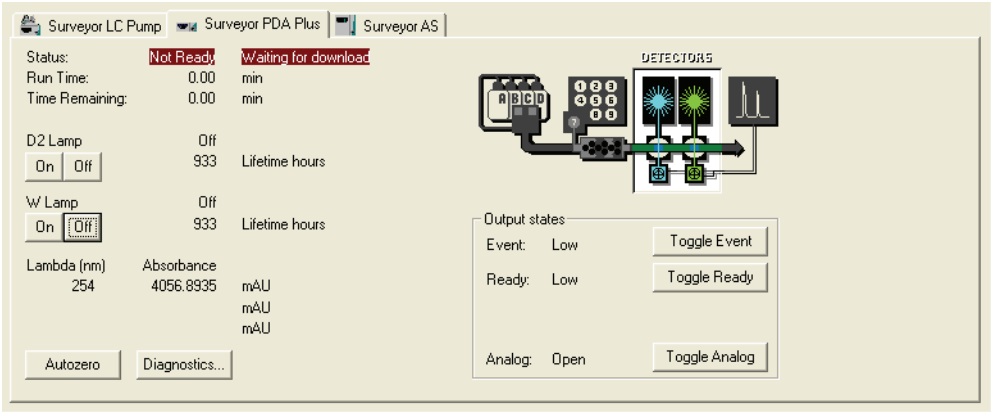
After you open the Instrument window, check the status of the instrument modules.

### ❖ To check the status of the modules

1. From the Instrument window menu bar, choose **Control > Instrument Status**.

   The Instrument Status window opens. See Figure 95. This dialog box contains one page for each configured module.

   **Figure 95.** Instrument Status window – Surveyor PDA Plus page




2. Click each page in the Instrument Status window and check the status readouts.

   If you have just turned on the power and have not yet downloaded a method, you see the following status readouts:

| Module | Status |
|---|---|
| UV/Vis detector | Ready or Lamps Off |
| PDA detector | Ready or Not Ready Waiting for Download |
| Pump | Idle |
| Autosampler | Waiting for Download |
| RI detector | Ready or Wait Temp |
| FL detector | Ready or Device Lamp Off |

3. If the lamps are Off, do one of the following depending on which detector you are running:

   - For the Surveyor PDA Plus Detector, click the D2 Lamp On button and the W Lamp On button. See Figure 95.
   - For the Surveyor UV/Vis Plus Detector, the lamps turn on when you download a method.

The deuterium lamp takes approximately 10 seconds to ignite. If you have a PDA detector, ensure that the lamps are turned on and ready.

- For the Surveyor FL Plus Detector, the xenon lamp turns on when you download a method.
- For the Surveyor RI Plus Detector, the tungsten lamp should always be On when the detector is turned on.

Go to the next topic, Removing Air from the Solvent Lines.

# Removing Air from the Solvent Lines

After you change the solvents in the solvent reservoir bottles or the wash bottle, your system will have air in the solvent lines. The solvent lines connecting the solvent reservoir bottles to the built-in vacuum degasser of the pump hold approximately 3 mL of solvent each [5-ft. *l* × 0.0625-in. ID (152 cm *l* × 0.159 cm ID)]. The wash bottle solvent line that connects the wash bottle to the syringe valve holds a similar volume of solvent.

Air in the solvent reservoir lines causes excessive pump pulsation, as well as non-reproducible retention times. Air in the wash bottle tubing or the syringe or both causes non-reproducible injection volumes.

Even if you have not just changed the solvents in the solvent reservoir bottles or the wash bottle, check the solvent lines of your system for air before you begin making injections. The solvent lines are permeable to air, which means that inevitably, over time, air bubbles form in the lines.

To remove air from your solvent lines, purge the pump. To remove air from the syringe, flush it.

**Note** ChromQuest contains a login security feature that allows the system administrator to assign privileges to users and groups. If this security feature is enabled, users can be denied administrative and/or instrument privileges. To access the direct commands menu for the autosampler, which contains the *Flush Syringe* command, you must have instrument privileges.

This section contains the following procedures:

- Purging the Pump
- Flushing the Syringe
- Purging the Flow Cell of the RI Detector

# Purging the Pump

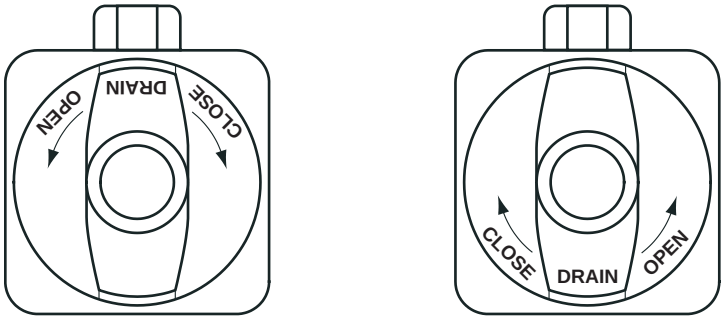
The purge command draws eluent from a solvent reservoir bottle at a rate of approximately 10 mL/min. As the solvent is drawn into the pump, it pushes air out of the solvent lines and the pump head assemblies.

### ❖ To purge the solvent lines

1. Open the drain valve by turning it counter-clockwise 180° to the purge position.

   The word DRAIN on the knob appears upside down as shown on the left in Figure 96.

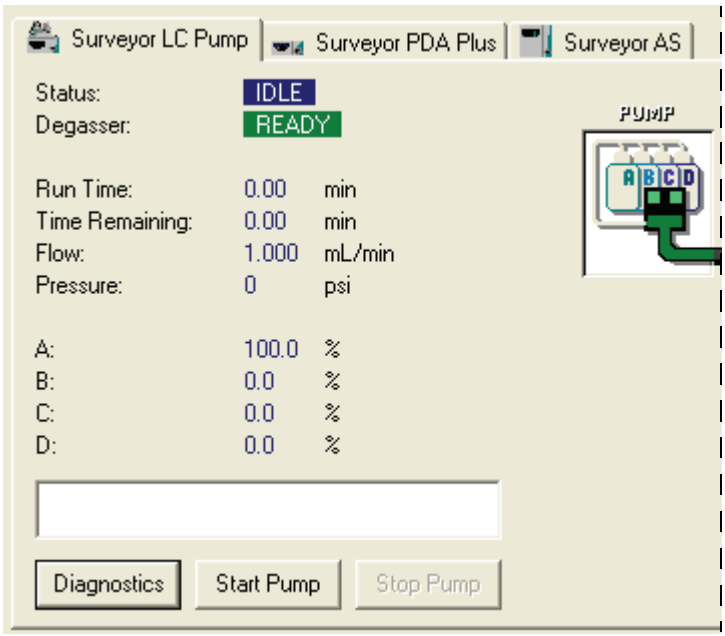
   **Figure 96.** Drain valve positions




   Open position  Closed position

2. Open the Diagnostics dialog box – Operation page for the LC pump:
   a. From the Instrument window menu bar, choose **Control > Instrument Status** to open the Instrument Status window.
   b. Click the **Surveyor LC Pump** tab to open the Surveyor LC Pump page. See Figure 97.

**Figure 97.** Instrument Status window – Surveyor LC Pump page

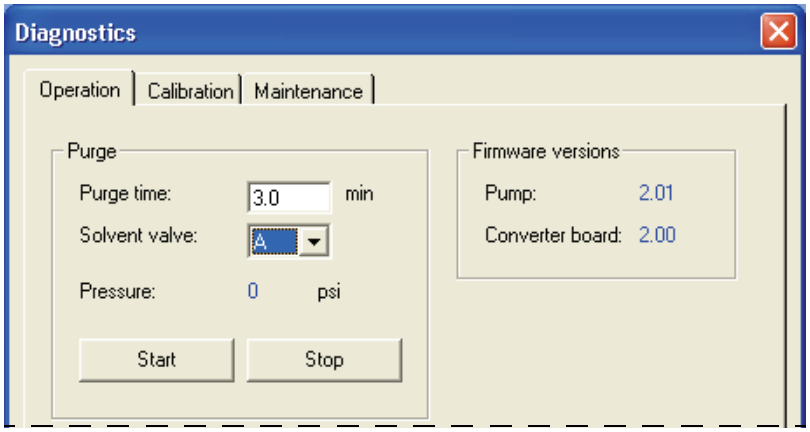



c. Click **Diagnostics**.

The Diagnostics dialog box – Operation page, shown in Figure 98, appears.

When the drain valve is open, the mobile phase flows to waste rather than to the column. Therefore, the backpressure of your system will be close to zero or zero.

**Figure 98.** Diagnostics dialog box – Operation page




3. Purge solvent lines A and B:

a. Under Purge, type **3** in the Purge time box.

b. In the Solvent valve list, select **A**.

c. Click **Start** to start purging the solvent line.

Each solvent line holds approximately 3 mL of solvent. In the purge mode, the pump draws solvent through the lines at a rate of approximately 10 mL/min. If there is a significant amount of air in a solvent line, you might need to purge the system for a longer period of time. Purge a solvent line until you flush all the air out of it.

**Note** If you generate a pump error (see Figure 99), ensure that you opened the purge valve as directed in step 1 of this procedure. To recover from the error, open the drain valve, close the Diagnostics dialog box, and then click **Reset Error** on Instrument Status window – Surveyor LC Pump page. See Figure 100.

**Figure 99.** Error message dialog box

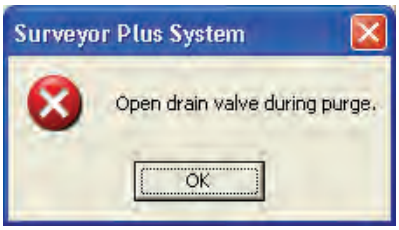
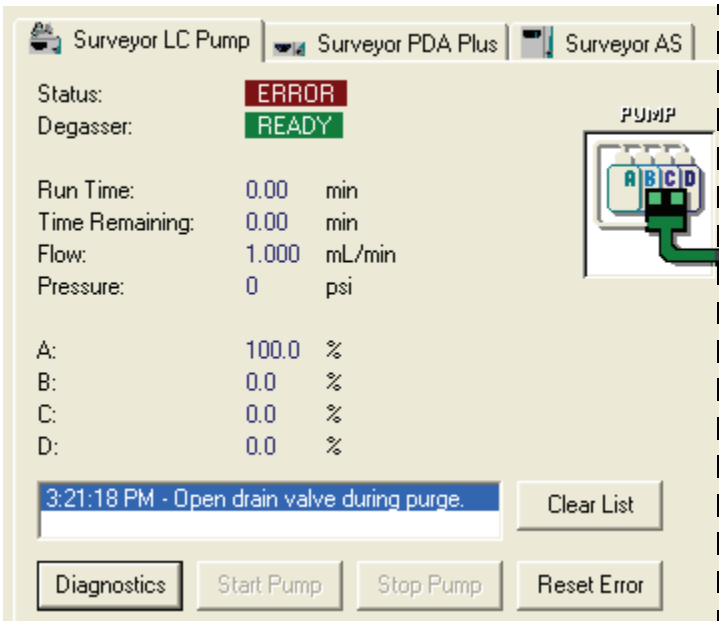



**Figure 100.** Error message read-only box




d. To purge solvent line B, repeat steps 3a to 3c, selecting **B** from the solvent valve list.

**Note** If the replacement solvent is not miscible with the current solvent in the line, flush the line with an intermediate solvent that is miscible with both the replacement solvent and the current solvent before flushing the line with the replacement solvent. If the current solvent is a buffered solution, purge the flow line with distilled water before you replace the solvent.

4. After you have finished purging the solvent lines of air, close the drain valve by gently turning it clockwise as far as it will go.

   **Note** Ensure that the drain valve is closed. If you do not close the drain valve, the mobile phase will continue to flow out through the waste line rather than to the autosampler.

5. Click **Close** to exit the Diagnostics dialog box.

## Flushing the Syringe

If the syringe or the wash bottle line contains a large quantity of air, the autosampler will not be able to withdraw sample from the sample vial. Therefore, before you attempt to make an injection, check the wash bottle line and the syringe for air. If you see air in the wash bottle line or the syringe, perform a **Flush Syringe** direct command to remove the air.

**Note** To flush the syringe, you must have Instrument privileges.

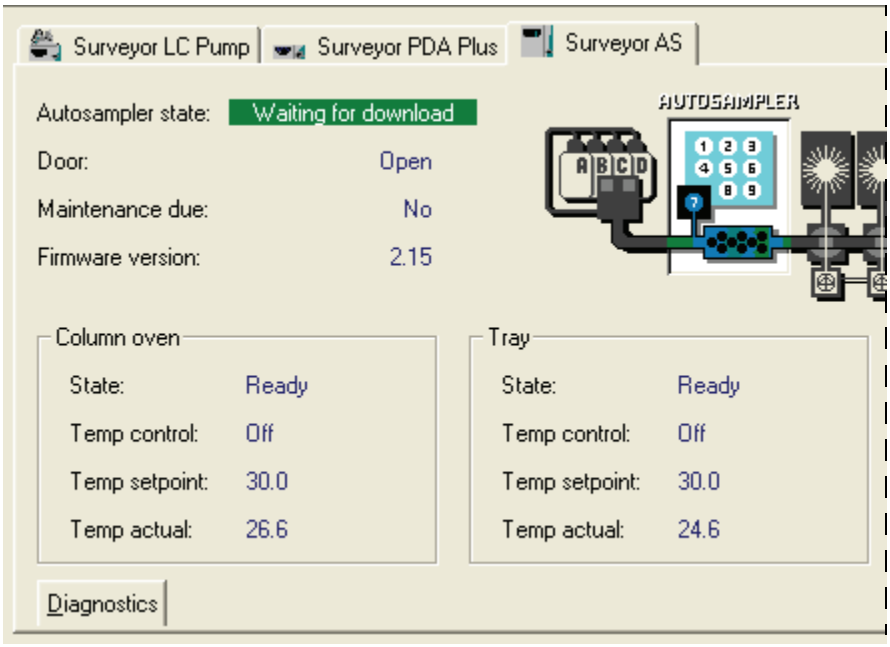
### ❖ To flush the syringe

1. Ensure that the wash bottle contains solvent.

   The wash bottle is contained in the solvent platform, located at the top of the Surveyor Stack. The wash bottle tubing is connected to the left side of the syringe valve.

2. Open the Direct Controls page of the Diagnostics dialog box:
   a. From the Instrument window menu bar, choose **Control > Instrument Status**.
   b. Click the **Surveyor AS** tab to open the Surveyor AS Instrument Status page, shown in Figure 101.

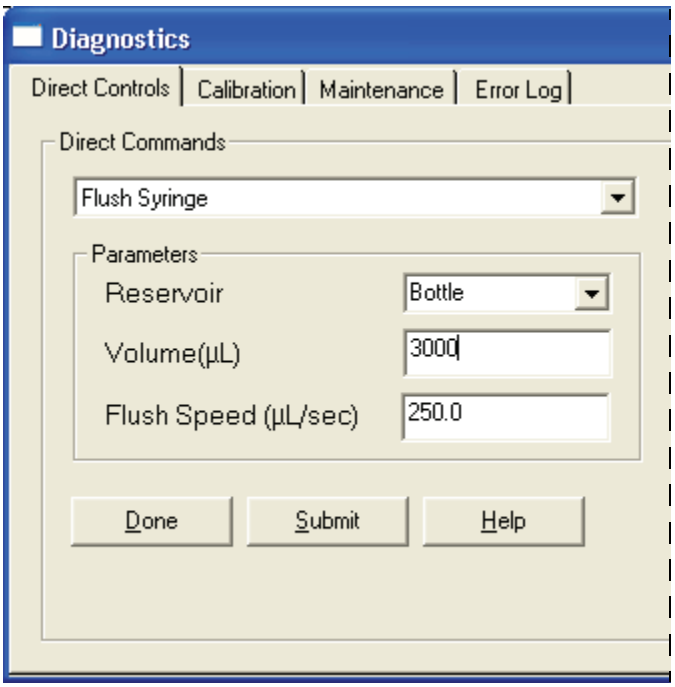
**Figure 101.** Instrument Status – Surveyor AS page




c. Click **Diagnostics**.

d. Click the **Direct Controls** tab to open the Direct Controls page, shown in Figure 102.

**Figure 102.** Direct Controls page, showing the selection of the Flush Syringe direct command



3. Perform a Flush Syringe command:

   a. In the Direct Commands list, select the **Flush Syringe** command.

      After you select the Flush Syringe command, the Parameters area below the list appears.

   b. In the Parameters area, select **Bottle** from the Reservoir list, and then type a value in the range from **100** to **6000** in the Volume box.

      The wash line holds approximately 3 mL of solvent. To replace the solvent in the line with new solvent, type a minimum value of 3000 µL.

      **Note** The default flush speed of 250 µL/s is only suitable for low viscosity solvents, such as 100% methanol. If you are using water or a water / methanol mixture for the flush solvent, lower the flush speed to 100 µL/s or less. If the flush speed is set too high, the syringe makes a grinding noise during the flush cycle and can stall.

      **Note** If the Verify Door is Closed feature is enabled, the Surveyor AS will not execute this command while the tray compartment door is open. To execute the command, close the tray compartment door, and then resubmit the command.

   c. Click **Submit** to execute the command.

   d. Click **Done** to exit Diagnostics.

      During a flush, the syringe needle is moved to the injection port of the autosampler to dispense a specified volume of solvent from the wash bottle or a reservoir vial. The injection valve is switched to the Inject position to prevent solvent from entering the sample loop. The flush solvent removes residual sample from the needle tubing assembly and the transfer tubing.

      The **Flush With Injector in Fill Position** direct command leaves the sample loop in the path of the wash solvent as you perform a flush.

# Purging the Flow Cell of the RI Detector

Use the purge control feature to remove air bubbles from the flow cell and to fill the reference compartment of the flow cell with the pre-mixed mobile phase specified in the method.

### ❖ To purge the flow cell of the RI detector

1. Start the pump flow:

   a. Choose **Method > Instrument Setup**.

   b. Click the **Surveyor LC Pump** tab.

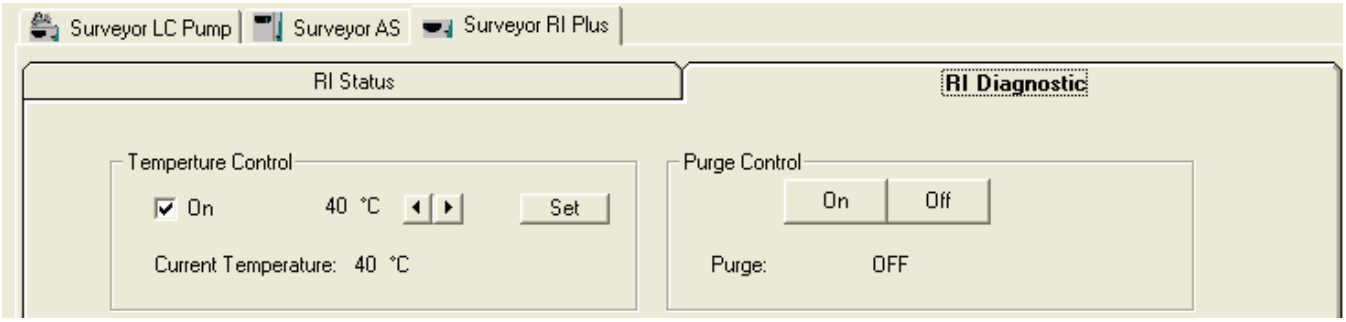
   c. Under Initial Settings, enter the appropriate flow rate and select the solvent bottle that contains your pre-mixed mobile phase.

d. Choose **Control > Download Tab** to download these parameters to the LC pump.

The pump flow starts. You can monitor a rise in the backpressure of the system by viewing the pressure readout in the Status area.

2. Begin flushing the flow cell:
   a. Choosing **Control > Instrument Status**.
   b. Click the **Surveyor RI Plus** tab.
   c. Click the **RI Diagnostic** tab.
   d. Under Purge Control, click **On**. See Figure 103.

**Figure 103.** RI Diagnostic page, showing the purge control buttons

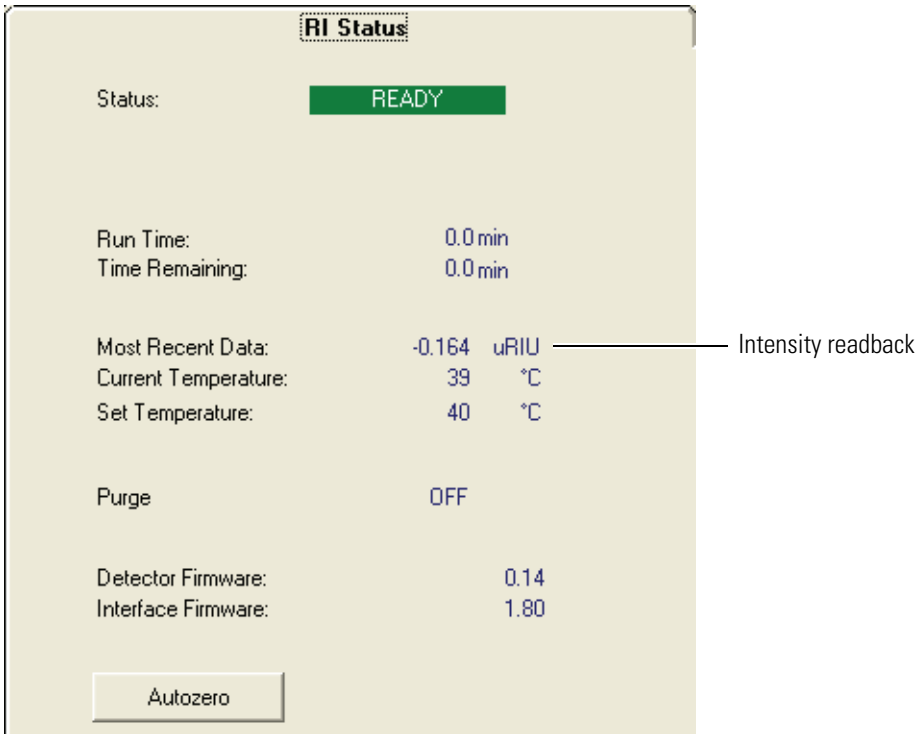



3. Pump the pre-mixed mobile phase at the set flow rate through the flowcell for 10 minutes.
4. To remove air bubbles, turn the purge control on for 10 seconds and then off for 10 seconds a few times.

   Purging is complete when the purge valve can be switched from **On** to **Off** with minimal baseline disturbance.
5. To view the baseline stability, click the **RI Status** tab to open the status page for the RI detector, and then monitor the rate of change of the most recent data. See Figure 104.

   Or, preview the baseline by choosing **Control > Preview** Run.

**Figure 104.** RI Status page, showing the Most Recent Data readback




# Downloading the Method

You added your instrument control parameters to the method when you performed the procedure "Creating an Acquisition Method" on page 81. For the new settings to take effect, you need to download the method to the instrument. Starting a single run automatically downloads a method. However, in general, you will probably want to download your method before your start a run so that you can equilibrate the LC column.

**Note** It requires approximately 15 to 20 times the unretained peak time of an LC column to equilibrate it. For reversed-phase chromatography, you can determine the unretained peak time of an LC column by injecting a non-retained analyte such as uracil or sodium nitrate, and then monitoring the absorbance at 254 nm or 210 nm for uracil or sodium nitrate, respectively.

After you download the method, the LC pump starts pumping mobile phase through the LC column. At a flow rate of 1 mL/min, it takes approximately 15 to 20 minutes to equilibrate the 10 cm, 5 micron particle size, Hypersil, C-18 column.

❖ **To download your method**

1. If the name of your method is not listed in the title bar of the Instrument window, open your method:
   a. From the Instrument window menu bar, choose **File > Method > Open**.
   b. Browse to the appropriate directory:
      Drive:\ChromQuest\Projects\Tutorial\Methods.
   c. Select your method and click **Open**.
   d. Verify that your method is listed in the title bar of the Instrument window.
2. Choose **Control > Download Method** from the Instrument window menu bar to download your method.

   The Analysis Channel list displays the analysis wavelengths contained in the method. The LC pump begins pumping the specified conditions.

   If your system contains a UV/Vis detector, the deuterium lamp ignites, and the Lamps LED turns green within 10 seconds.

   If your system contains an RI detector and the method specifies an operating temperature, the RI detector equilibrates to the set temperature. Before beginning an analysis that requires maximum sensitivity, allow the RI detector to equilibrate at the set temperature for 24 hours.

   If you did not close the purge valve after you purged the solvent lines, a below minimum pressure pump error appears when the pump attempts to stabilize the solvent flow.
3. If the below minimum pump error message appears, do the following:
   a. Close the purge valve of the pump.
   b. Open the Instrument Status page for the Surveyor LC Pump.
   c. Click Reset Error.

# Checking the Stability of the Baseline

There are two features in ChromQuest that allow you to check the stability of the baseline: Preview Run and Baseline Check. The Preview Run feature allows you to visually check the stability of the baseline. In addition to a visual check, the Baseline Check feature determines the noise and drift of the baseline and allows you to delay a run until these parameters meet the specified test criteria. To familiarize yourself with these features perform the following procedures:

- Previewing the Baseline
- Performing a Manual Baseline Check

## Previewing the Baseline

You can visually inspect the stability of the baseline by previewing the run.

### ❖ To preview the baseline for both wavelengths

1. Choose **Control > Preview Run**.

   After you initiate a preview run, the following messages appear sequentially in the status bar at the bottom of the Instrument window:

   Loading Method

   Creating Data File

   Downloading Method

   Equilibrating Method

   Running Sample <None>

   The data file name for the preview run, Instrument # Preview.dat, is listed in the Title bar of the Instrument window.

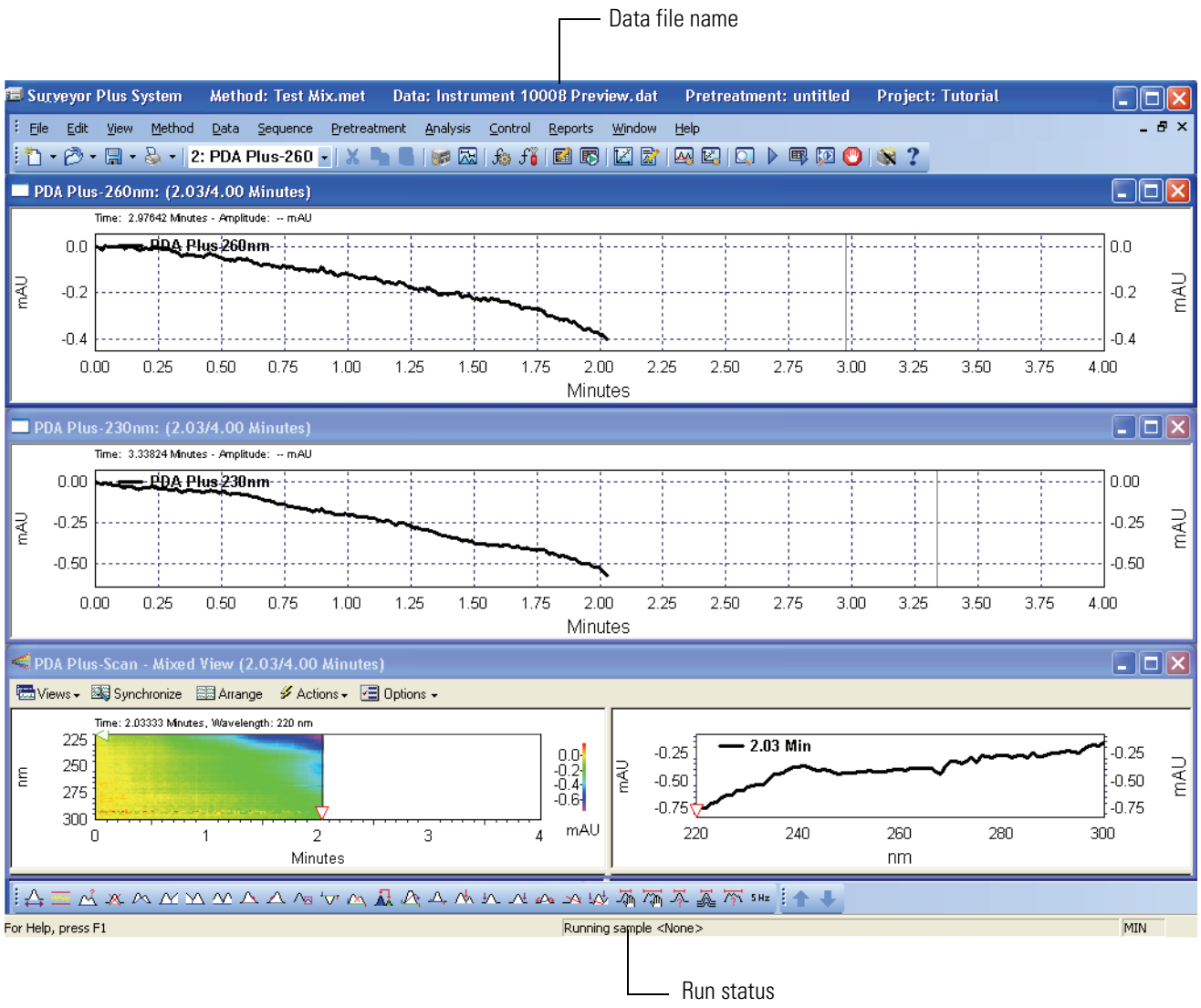
2. Choose **View > Tile Data** to tile the two chromatograms on your view screen.

3. Choose **Windows > Tile Horizontally** to tile the chromatograms horizontally on the view screen.

   If you created the method, Test Mix.met, while performing the procedure "Creating an Acquisition Method" on page 81, the two chromatograms are for wavelengths 230 nm and 260 nm. See Figure 105.

4. Click the **Stop Run**  button to stop the preview run, and then wait for the run to end.

**Figure 105.** Preview run screen for Test Mix method



## Performing a Manual Baseline Check

Before you make your first injection, perform a manual baseline check to confirm that the baseline is stable and to test the baseline for excessive drift.

### ❖ To perform a manual baseline check

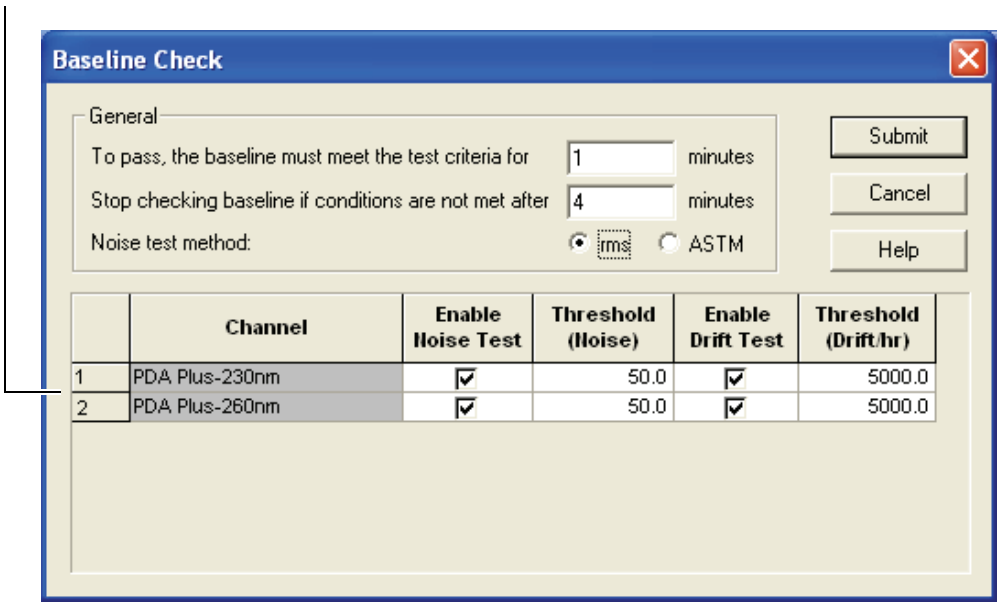
1. From the Instrument window, choose **Control > Baseline Check** to open the Baseline check dialog box.

   If the Baseline Check option is not available, the Baseline Check check box was cleared when your instrument was configured. Skip this procedure.

2. Keep all parameters in the Baseline Check dialog box set to the default settings except those that are shown in the following table and in Figure 106.

| Parameter | Setting | Result |
|---|---|---|
| General | | |
| • To pass, the baseline must meet the test criteria for | 1 | Specifies that the baseline must pass the test criteria for a time interval of 1 min |
| • Stop checking baseline if conditions are not met after | 4 | Specifies that if the baseline has not passed the test criteria for the length of time specified (1 min), then the program will discontinue the test after a time interval of 4 min and report a failing result |
| Test Criteria | | |
| • PDA-230 nm Enable Noise Test | ☑ | Specifies that a noise test will be performed for the 230 nm wavelength channel |
| • Threshold (Noise) | 50 | The rms noise must be < 50 µAU. |
| • PDA-230 nm Enable Drift Test | ☑ | Specifies that a drift test will be performed for the 230 nm wavelength channel. |
| • Threshold Drift | 5000 | The drift must be < 5 mAU/hr (< 83 µAU/min) to pass. |
| • PDA-260 nm Enable Noise Test | ☑ | Specifies that a noise test will be performed for the 260 nm wavelength channel. |
| • Threshold (Noise) | 50 | The rms noise must be < 50 µAU. |
| • PDA-260 nm Enable Drift Test | ☑ | Specifies that a drift test will be performed for the 260 nm wavelength channel. |
| • Threshold Drift | 5000 | The drift must be < 5 mAU/hr (< 83 µAU/min) to pass. |

**Figure 106.** Baseline Check dialog box



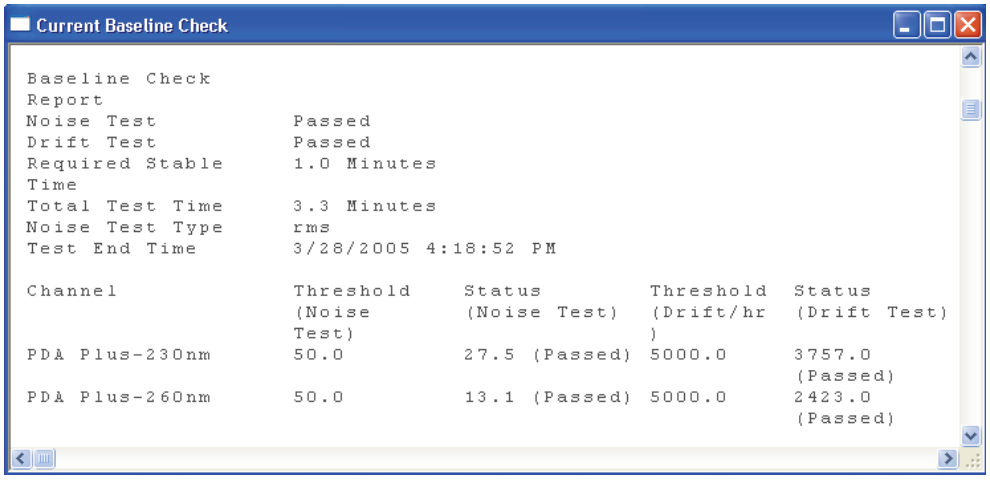

3. Click **Submit**.

   The Baseline Check window appears.

4. If the Baseline Check window does not appear, from the Instrument window menu bar, choose **View > Baseline Check**.

5. To print a Baseline Check report (see Figure 107) after the baseline check is complete, choose **Reports > View > Current Baseline Check**.

**Figure 107.** Baseline Check Results



# Making Your First Injection

In this tutorial, you learn how to load a sample into the tray compartment of the autosampler; start, extend, and stop a single run; view the real-time progress of a run; manually check the purity of your chromatographic peak; and add annotations to your chromatograms.

Before you perform this tutorial, confirm that you have done the following:

- Configured your Surveyor Plus HPLC instrument
- Filled the solvent bottles for the mobile phase and the wash solvent
- Removed the air from the solvent lines
- Downloaded an acquisition method, warmed up the lamps of the detector, and equilibrated the LC column
- Checked the stability of the baseline

If your instrument has not been configured, see Chapter 3, "Configuring Your Instrument." If you have not created an acquisition method, see Chapter 4, "Creating Methods." If your instrument is not ready to perform a run, see Chapter 5, "Preparing Your Instrument for a Run."

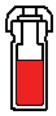
**Contents**

# Loading the Sample into the Tray Compartment

A set of ampules labeled Autosampler Test mix is supplied in the accessory kit for the autosampler. This test mix contains a solution of 0.5% toluene in methanol. If your system contains a UV/Vis or PDA detector, use this test mix to make your first injection. If you do not have an ampule of this test mix solution or your system contains an RI or FL detector, inject an analyte* for which you know the chromatographic conditions, and adjust the instrument setup section of the method accordingly.

**Note** Ensure that your sample(s) are completely soluble in the mobile phase and that you have filtered your sample(s) and solvents through a 0.5 micron filter. These techniques minimize sample precipitation in the lines and remove any particulate matter that could obstruct the flow through the injection port of the autosampler, the transfer tube connecting the injection port to the injection valve, and the injection valve itself.

### ❖ To load a sample vial into the tray compartment of the autosampler



1. Fill a standard 1.8 mL autosampler vial with the sample that you want to inject.
2. Open the Online Instrument window for your instrument:

   - For ChromQuest, if you are currently working in the Offline Instrument window, choose **Window > Main Menu**. The Main Menu window appears. Double-click the icon for your instrument to open the Online Instrument window. If you have enabled the instrument login and project management feature, (see Chapter 2, "Administrating the Enterprise," ) log in to the Tutorial project.
   - For ChromQuest SI, from the computer desktop, choose **Start > All Programs > Chromatography > ChromQuest SI**.
3. Open the left door of the autosampler.

   If you performed the tutorial contained in Chapter 3, "Configuring Your Instrument," which instructed you to clear the Verify door is closed option for the autosampler, the XYZ arm of the autosampler will not move to the back of the tray compartment when you open the door.

   **Note** ChromQuest contains a login security feature that allows the system administrator to assign privileges to users and groups. If this security feature is enabled, users can be denied administrative and/or instrument privileges. To access the direct commands menu for the autosampler, which contains the *Position Arm to Remove Tray* command, you must have instrument privileges.
4. Perform the **Position Arm to Access Tray** direct command to move the XYZ arm to the back of the tray compartment:
   a. From the Online Instrument window, choose **Control > Instrument Status**.
   b. Click the **Surveyor AS** tab to open the Instrument Status window – Surveyor AS page.

c. Click **Diagnostics** to open the Diagnostics dialog box for the autosampler.

d. From the Direct Commands list, select **Position Arm to Access Tray**.

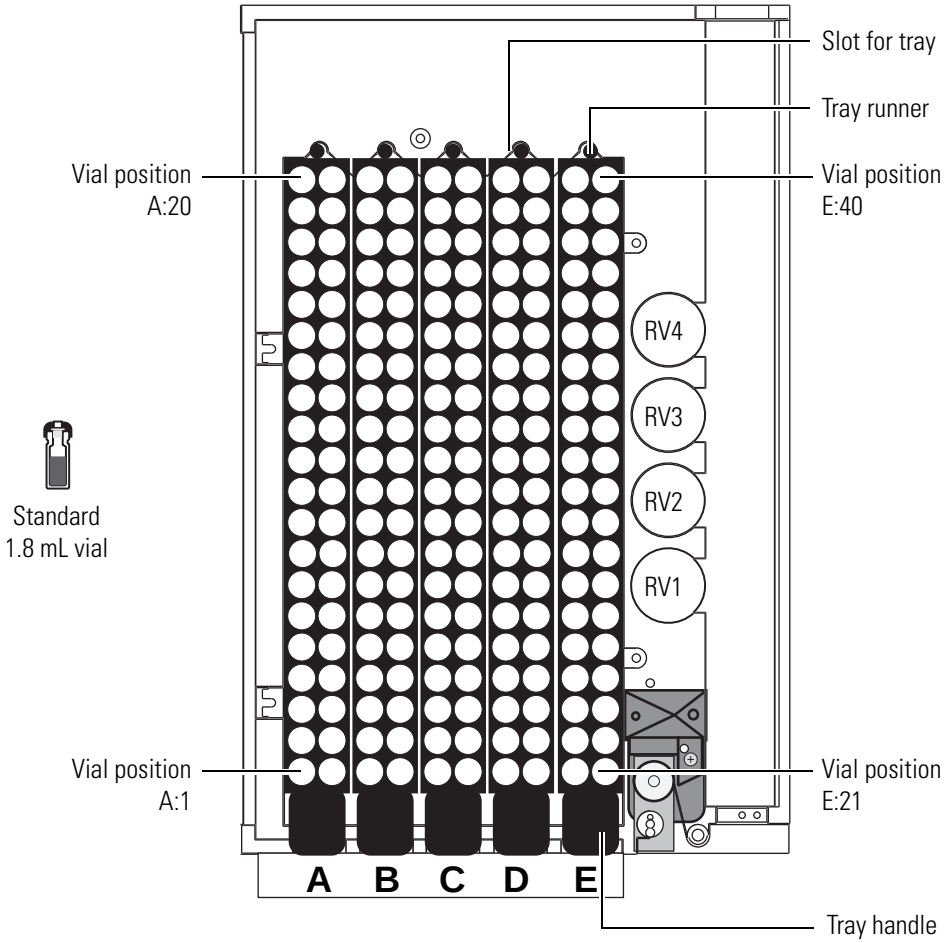
e. Click **Submit**.

The XYZ arm moves to the back of the tray compartment.

5. Place the vial in position A1 of a standard tray. Then, load the tray into the autosampler tray compartment. If the tray is already loaded in the tray compartment, place the vial in position A1. See Figure 108.

**Note** If the autosampler configuration option, Verify Door is Closed, is enabled the autosampler arm will not move to the requested vial position until the tray compartment door is closed.

**Figure 108.** Tray compartment, showing five standard trays installed



# Starting, Extending, and Stopping a Single Run

Before you can start a single run, you must create an acquisition method containing the appropriate instrument control parameters. If you performed the tutorial in Chapter 4, "Creating Methods," you created the method, Test Mix.met.

### ❖ To start a single run

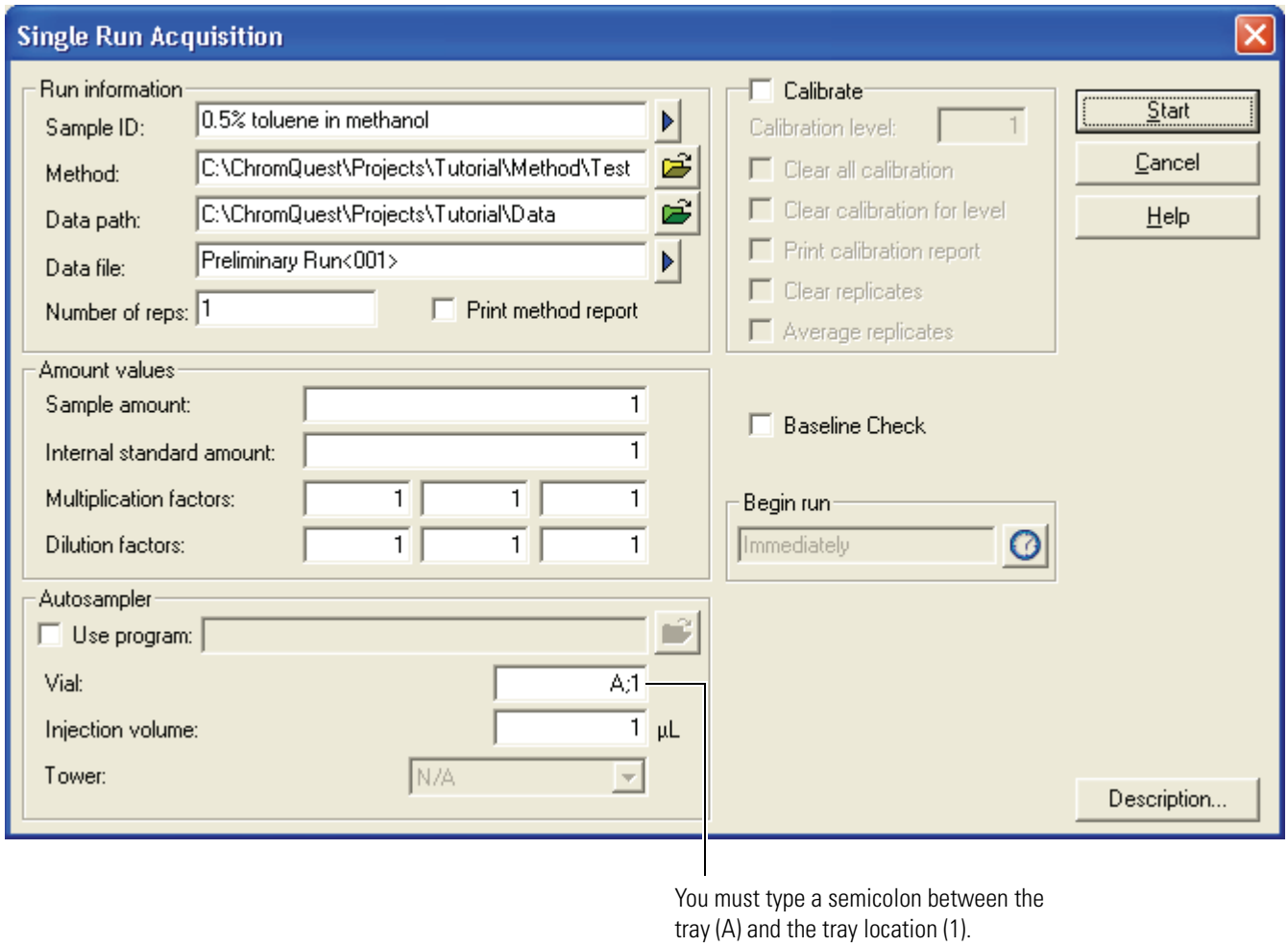
1. Ensure that the method, Test Mix.met, is listed in the title bar of the Instrument window. If the appropriate method is not listed, open it:
   a. From the menu bar, choose **File > Method > Open**.
   b. Browse to the appropriate directory: Drive:\ChromQuest\Projects\Tutorial\Method.
   c. Select the method, Test Mix.met.
   d. Click **Open**.
2. In the Instrument window toolbar, click the **Single Run** button to open the Single Run Acquisition dialog box.
3. Keep all parameters in the Single Run Acquisition dialog box set to the default settings except those that are shown in the following table and in Figure 109. The active method will be listed in the Method box.
4. To create the data file name listed in the table below, type **Preliminary Run** in the Data File box. Then click the blue arrow to the right of the Data file box to open a shortcut menu and choose **Increment Number**.

| Parameter | Setting | Result |
|---|---|---|
| Run Information | | |
| • Sample ID | 0.5% Toluene in methanol | Gives the data file a sample ID, which you can use with the search feature in ChromQuest. |
| • Data Path | Drive:\ChromQuest\Projects\Tutorial | ChromQuest stores the data file in this folder. |
| • Data File | Preliminary Run <001> | Names the data file as Preliminary Run 001.dat. ChromQuest adds the .dat file extension to data files. |

| Parameter | Setting | Result |
|---|---|---|
| Autosampler | | |
| • Vial | A;1 | The autosampler withdraws sample from vial location A1. You must type a semicolon between the tray (A) and the tray location (1). |
| • Injection Volume | If the detector contains a 5 cm LightPipe flowcell, type **1**.<br><br>If the detector contains a 1 cm flowcell, type **5**. | The autosampler meters 1 µL of sample into the sample loop.<br><br>The autosampler meters 5 µL of sample into the sample loop. |

**Note** The LightPipe flowcell has five times the sensitivity of a standard 1 cm flowcell. Therefore, if your detector contains a 5 cm LightPipe flowcell, you can inject one-fifth the sample volume to achieve the same absorbance level as you would achieve using a standard 1 cm flowcell.

**Figure 109.** Single Run Acquisition dialog box with entries




You must type a semicolon between the tray (A) and the tray location (1).

**Note** Ensure that the check box to the left of the Use Program box is not selected. If you leave this check box selected, the autosampler attempts to run a pretreatment method before it injects the sample.

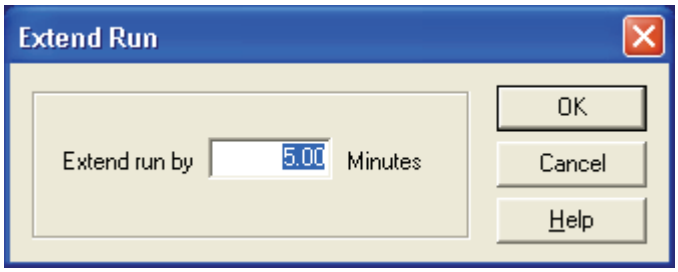
5. Click **Start.**

The following steps are included in this procedure to introduce you to the Extend Run and Stop Run features in ChromQuest.

6. After 3 minutes into the run, choose **Control > Extend Run.**

   The Extend Run dialog box appears, with a default time of 5 minutes in the Extend run by box.

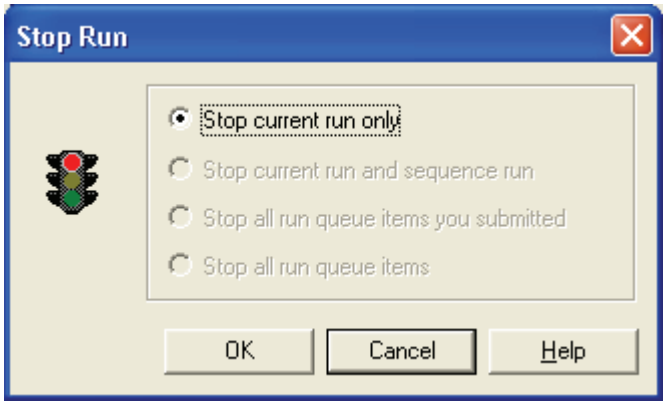
**Figure 110.** Extend Run dialog box




7. Click **OK** to extend the run by five minutes.
8. Before the additional time has elapsed, click the **Stop Run** button in the Command toolbar to stop the run.

   The Stop Run dialog box, shown in Figure 111, appears.

**Figure 111.** Stop Run dialog box




9. Click **OK** to stop the run.

   By default, the chromatograms are analyzed after the data file is acquired. After you stop a run, ChromQuest stores the data file without analyzing it. To change the frequency at which ChromQuest analyzes data, use the Options page of the Method Properties dialog box.
10. Because you stopped the run, you must click the **Analyze** button in the Command toolbar to integrate the chromatograms.

Table 9 lists the sequence of events for a partial loop injection.

**Table 9.** Sequence of events for a partial loop injection

| Step | Events |
|---|---|
| Draw transport solvent | The autosampler switches the two-way syringe valve to the wash line position.<br><br>The inner plunger of the concentric syringe moves downward, drawing solvent from the wash bottle. The amount of solvent drawn is equal to the dead volume of the autosampler plus 7.5 µL. |
| Draw sample | The XYZ arm moves to the requested sample vial.<br><br>The autosampler switches the two-way syringe valve to the needle tube position.<br><br>The inner plunger of the concentric syringe moves downward, drawing 3 µL of air into the needle.<br><br>The XYZ arm lowers the needle into the sample vial.<br><br>The inner plunger of the concentric syringe moves further downward, drawing the requested sample volume of 1 µL plus an additional 22 µL into the needle tubing.<br><br>The XYZ arm raises the needle out of the sample vial.<br><br>The inner plunger of the concentric syringe moves further downward, drawing an additional 3 µL of air into the needle, effectively isolating the sample with air. |
| Push transport solvent | The XYZ arm moves to the injection port, and then lowers the needle into the injection port.<br><br>The Inner plunger of the concentric syringe moves upward, pushing the sample out of the needle and into the transfer tubing that connects the injection port of the autosampler to the injection port (port 2) of the injection valve. The inner plunger of the concentric syringe continues to move upward until approximately half of the excess sample is pushed out to waste through port 3 of the six-port injection valve. |
| Load sample into loop | The autosampler switches the six-port injection valve to the fill position, and then meters 1 µL of sample (requested injection volume) into the front of the sample loop (through port 1).<br><br>If you listen carefully, you can hear the valve switch to the fill position. |
| Inject sample | The autosampler switches the injection valve to the inject position, allowing the mobile phase stream to backflush the sample onto the column (mobile phase enters the sample loop through port 4).<br><br>The inner plunger of the syringe continues to move upward, expelling excess sample to waste.<br><br>Again, if you listen, you can hear the valve switch. The injection valve remains in the inject position for the remainder of the run, allowing the mobile phase to thoroughly flush the sample loop. |

# Viewing the Progress of Your Run

After you begin your first injection, you can view the real-time progress of the run on your view screen. The viewing options that are available to you depend on the type of data you are collecting. If you using the UV/Vis detector to collect dual-wavelength data files, you are able to simultaneously view the collection of both chromatograms. If you are collecting discrete channels and scan data using the PDA detector, you are able to view the discrete channel chromatograms as well as the spectral data.

This section contains the following topics:

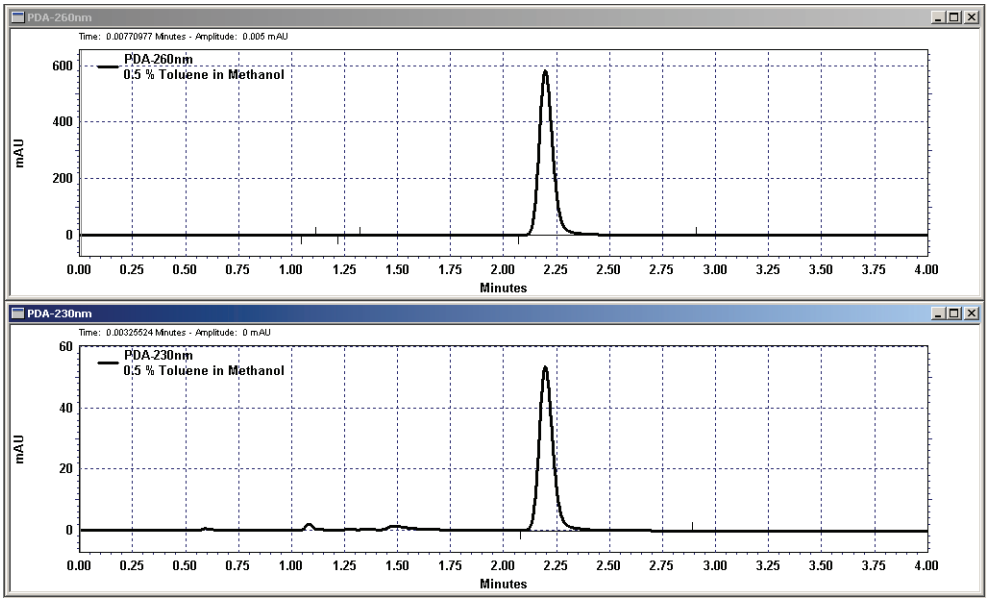


## Viewing Your Chromatograms

The chromatograms can be viewed in separate windows or overlaid in one window. If you are viewing the chromatograms in separate windows, the windows can be tiled horizontally or vertically on the screen.

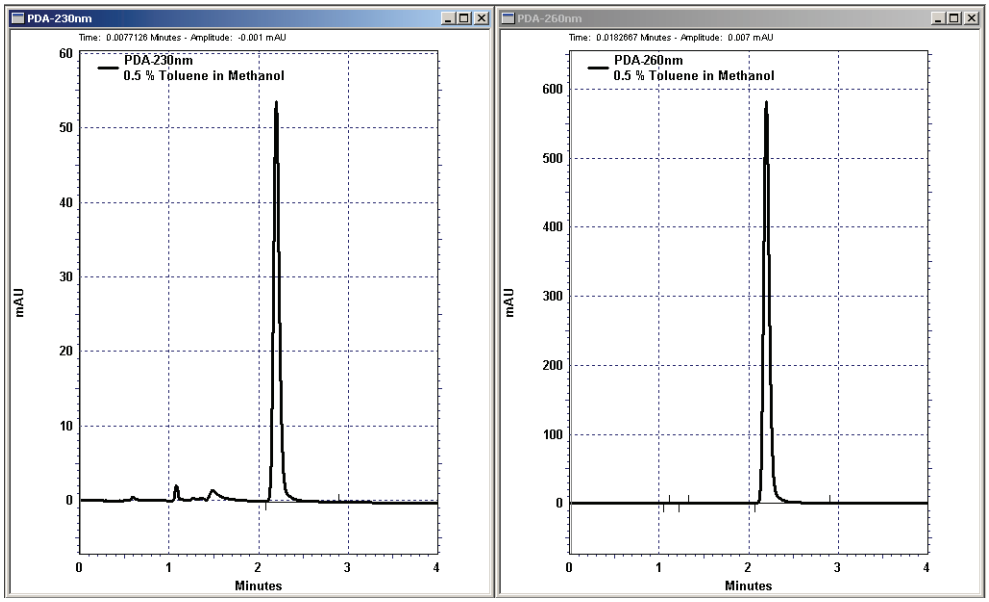
- To overlay the chromatograms, choose **View > Overlay**.
- To tile the Chromatogram windows, choose **View > Tile Data**.
- To tile the Chromatogram windows horizontally, choose **Window > Tile Horizontally**. See Figure 112.

**Figure 112.** Horizontally tiled chromatograms



- To tile the Chromatogram windows vertically, choose **Window > Tile Vertically**. See Figure 113.

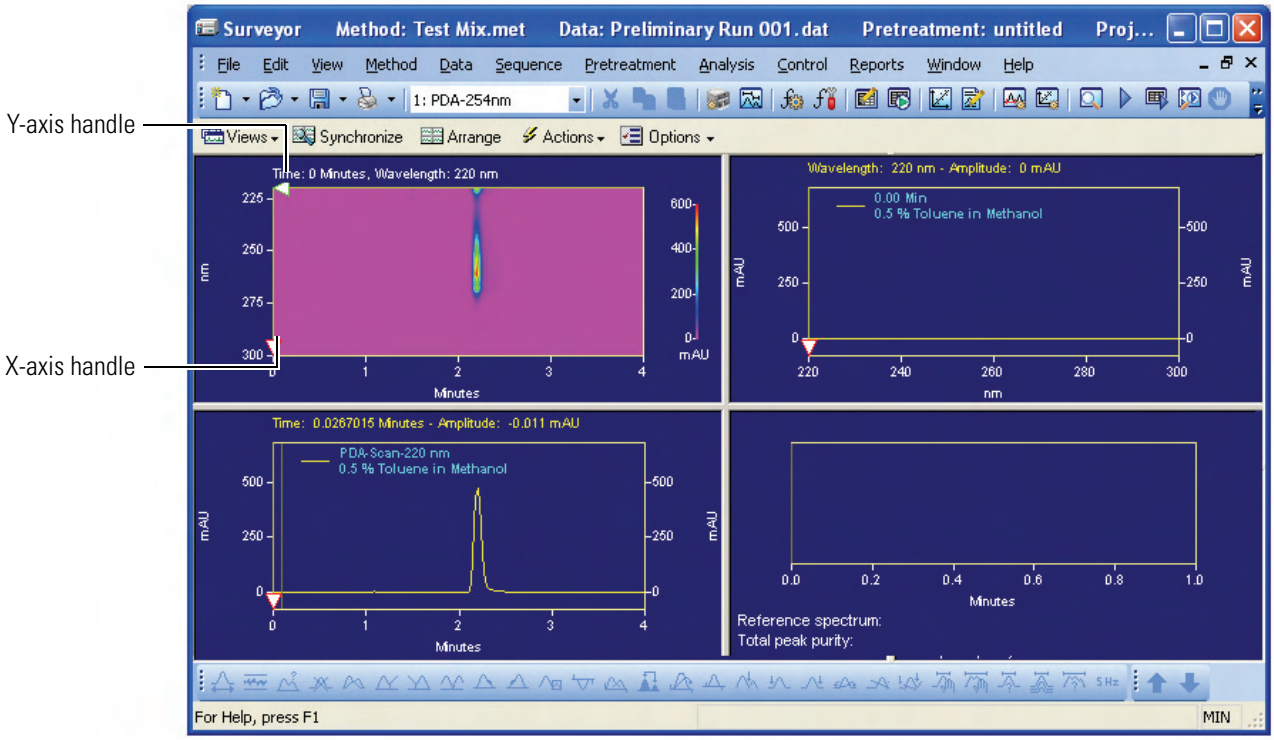
**Figure 113.** Vertically tiled chromatograms



## Viewing Your Spectral Data

There is a separate submenu for viewing scan data. If you are using the PDA detector to collect a scan data file, choose **View > Spectral View > Mixed View** to open the view shown in Figure 114. This view contains four tiled window panes. The upper left quadrant contains the Contour Plot pane. The lower left quadrant contains the Chromatogram pane. The upper right quadrant contains the Spectrum pane. The lower right quadrant contains the Peak Purity pane. Notice the two handles on the contour plot. The handle pointing to the 220 nm wavelength is the Y-axis handle. The handle pointing to the 0 min time point is the X-axis handle.

**Figure 114.** Instrument window, showing Mixed View



### ❖ To view the chromatogram for a specific wavelength

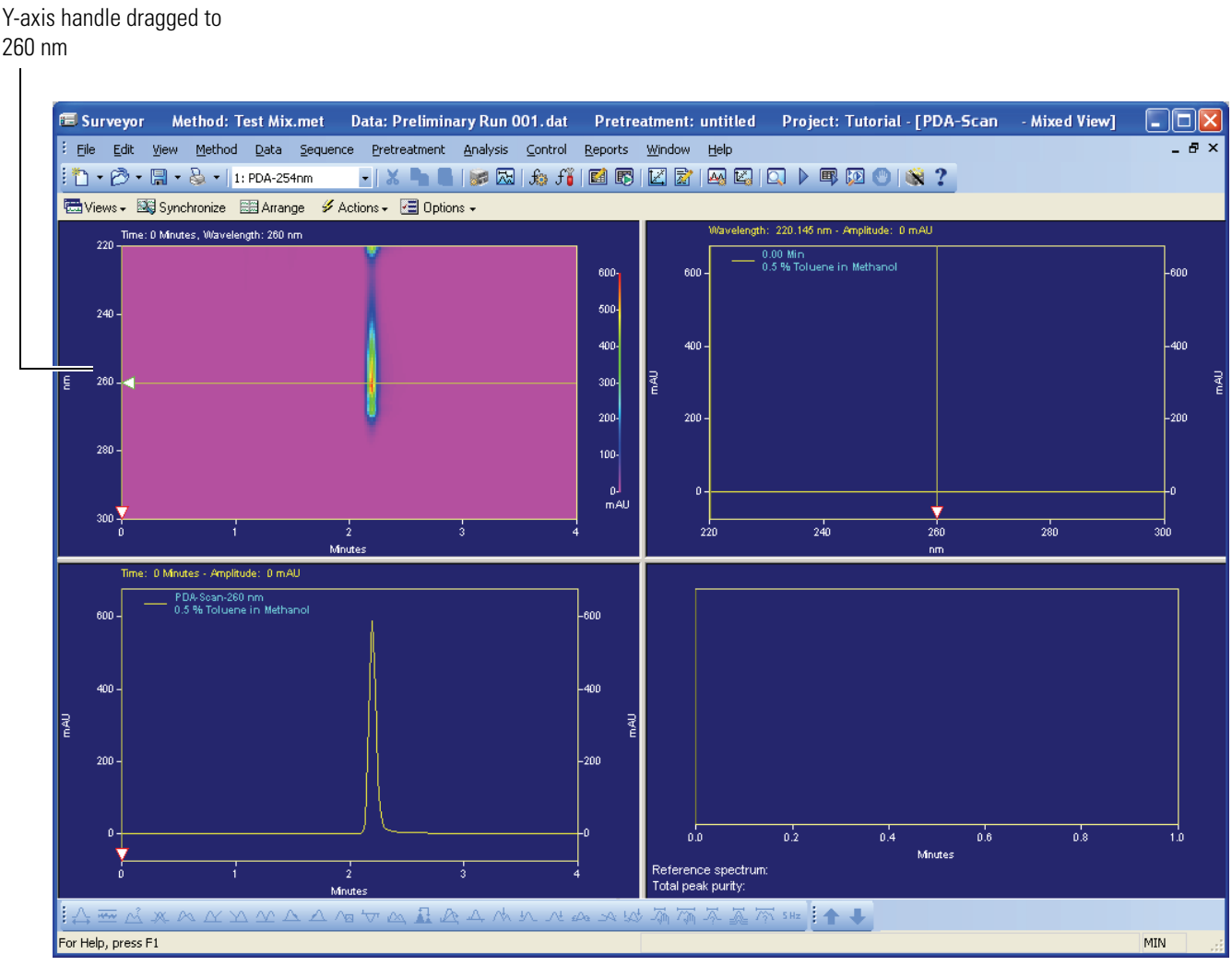
1. Click the Y-axis handle on the contour plot.

   The arrow at the left end of the handle points to the wavelengths on the Y-axis of the contour plot.

2. Drag the handle down from the 220 nm wavelength to the 300 nm wavelength. As you drag the handle down the Y-axis, notice how the Chromatogram window changes to display the chromatogram for the selected scan wavelength.

   See Figure 115, which shows the Y-axis handle pointing to the 260 nm scan wavelength.

**Figure 115.** Instrument window, showing the Y-axis handle pointing to the 260 nm scan wavelength



### ❖ To view the spectrum for a specific time point

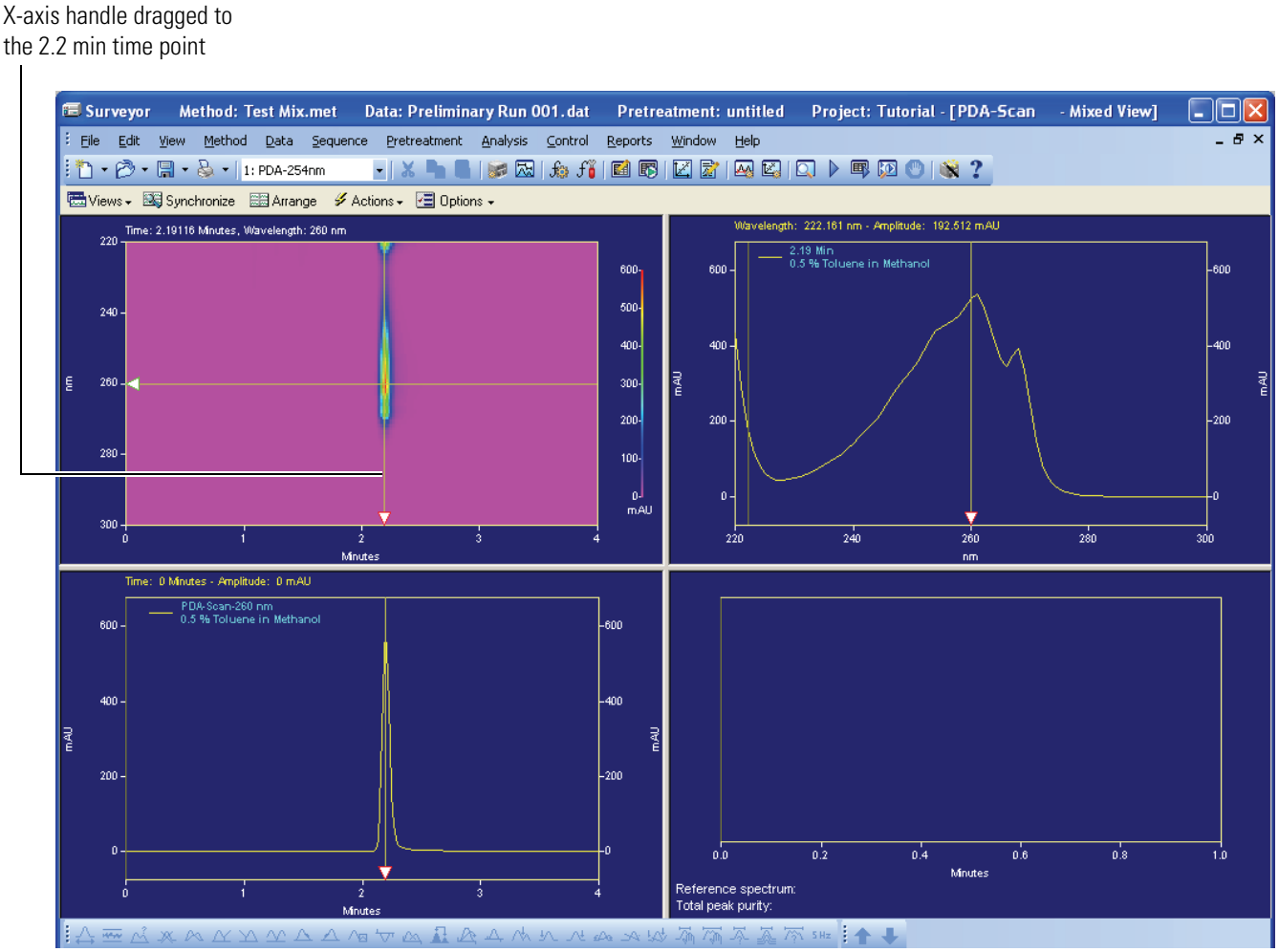
1. Click the X-axis handle on the contour plot.

   The arrow at the bottom of the handle points to the time on the X-axis of the contour plot.

2. Drag the handle across the peak for toluene and notice how the display in the Spectrum window changes.

   See Figure 116, which shows the X-axis handle pointing to the 2.20 min time point.

**Figure 116.** Instrument window, showing the X-axis handle pointing to the 2.20 min time point



# Checking the Purity of the Toluene Peak

If you have collected scan data with the PDA detector, you can determine the purity of the chromatographic peaks.

ChromQuest determines the purity of a peak by calculating the similarity of the spectra taken across the entire peak or by calculating the similarity of the spectra from the beginning, peak apex, and end of the peak. In both cases, peak purity is a measure of the homogeneity of the spectral slices that are being compared. If two or more compounds perfectly co-elute, ChromQuest reports a peak purity value of 1.0 (or close to 1.0) even though the peak is impure.

After you acquire a data file, perform the following procedures to check the purity of your toluene peak:

- Enabling Spectrum Calculations
- Performing a Manual Peak Purity Check

## Enabling Spectrum Calculations

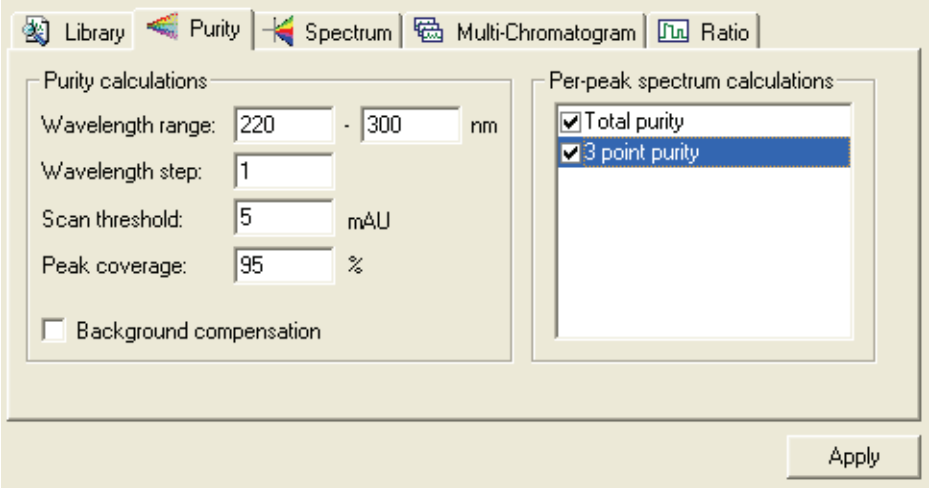
To check the purity of your peaks, your must enable spectrum calculations in the method.

### ❖ To enable spectrum calculations

1. From the Instrument window, choose **Method > Spectral Options** to open the Spectral Options window.
2. Click the **Purity** tab to open the Purity page.
3. Keep all parameters in the Purity page set to the default settings except those that are shown in the following table and in Figure 117.

| Parameter | Setting | Result |
|---|---|---|
| Purity Calculations | | |
| • Wavelength Range | 220 - 300 | Specifies that the spectra are taken from the wavelength range of 220 to 300 nm |
| Per Peak Spectrum Calculations | | |
| • Per Peak Spectrum Calculations | ☑ Total Purity | Enables the total purity calculation |
| • Per Peak Spectrum Calculations | ☑ 3-Point Purity | Enables the 3-point purity calculation |

**Figure 117.** Spectral Options window – Purity page




4. Click **Apply**.
5. Close the Spectral Options window.
6. Save the method by choosing **File > Method > Save**.

## Performing a Manual Peak Purity Check

The purity of the chromatographic peaks can be checked manually or automatically. For instructions on performing automated peak purity checks, see the online Help in ChromQuest.

### ❖ To manually check the purity of the toluene peak

1. Drag the Y-axis handle in the contour plot to the 260 nm wavelength.

   The unintegrated chromatogram for the 260 nm scan wavelength appears in the chromatogram window (see Figure 115). An unintegrated chromatogram can be distinguished by its lack of a baseline.

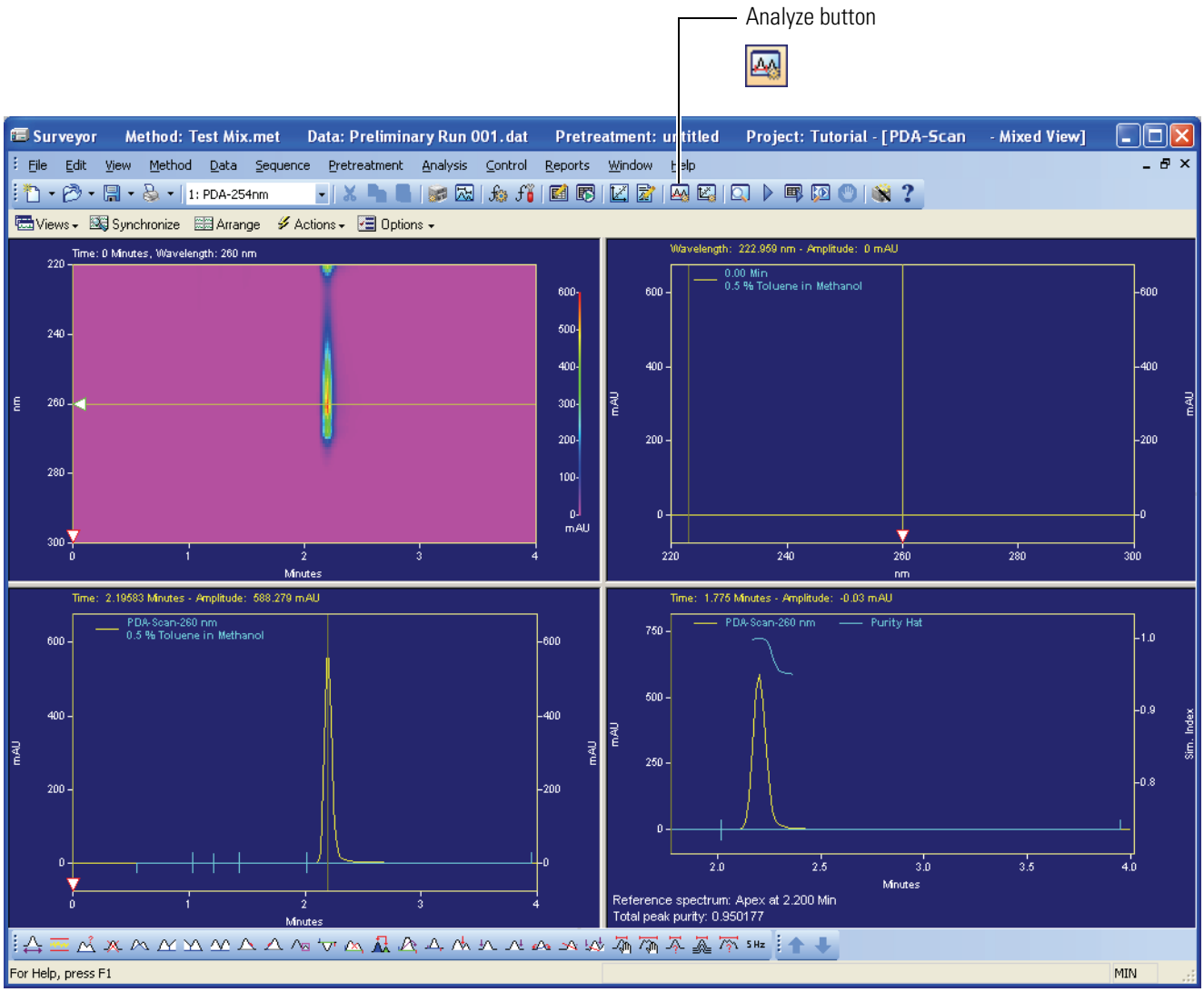
2. In the Instrument window toolbar, click the **Analyze** button to integrate the chromatogram.

   After you click the Analyze button, a chromatographic baseline appears.

3. In the Chromatogram window of the Mixed View, hold down the **CTRL** key, and then click the toluene peak.

   A peak purity plot appears in the Peak Purity pane of the Mixed View window, as shown in Figure 118.

**Figure 118.** Instrument window, showing the purity plot for the toluene peak in the bottom-right quadrant



# Adding Annotations to Your Chromatograms

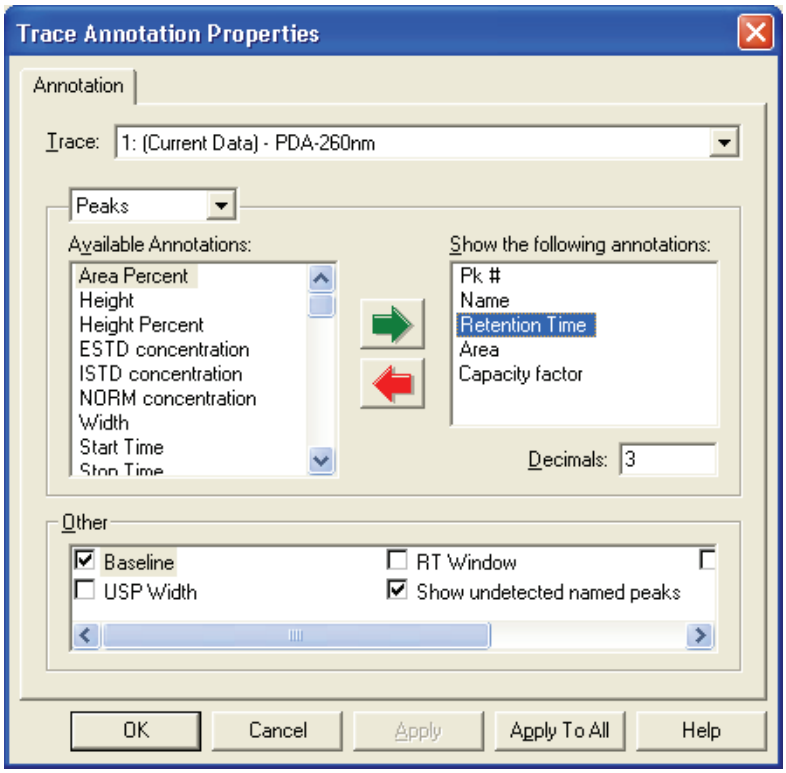
Now that you have collected your first data file, you are ready to add annotations to the chromatograms. In this tutorial, you add the following annotations: peak number, retention time, area, and capacity factor. Capacity factor is a performance parameter that uses the column information entered in the Advanced Method Options section of the method. If your method does not contain column information, ChromQuest reports a value of zero for any performance parameter annotation. For instructions on entering the information for your column to the method, see "Entering the Column Parameters" on page 95.

### ❖ To add annotations to your chromatograms

1. Choose **Window > Tile Vertically** to tile the discrete channel chromatograms on the view screen.
2. Right-click a chromatogram (alternatively referred to as a trace in ChromQuest) and choose **Annotations** to display the Trace Annotation Properties dialog box. See Figure 119.
3. In the Available Annotations list, double-click the following annotations:
   - Pk#
   - Retention Time
   - Area
   - Capacity Factor
4. In the Show the Following Annotations list, select **Retention Time**.

   The Decimals box is enabled and contains the default value of 3, as shown in Figure 119.
5. In the Decimals box, change the number of decimals to **2**:
   a. Highlight the number 3.
   b. Type **2** in the Decimals box.

**Figure 119.** Trace Annotations Properties dialog box



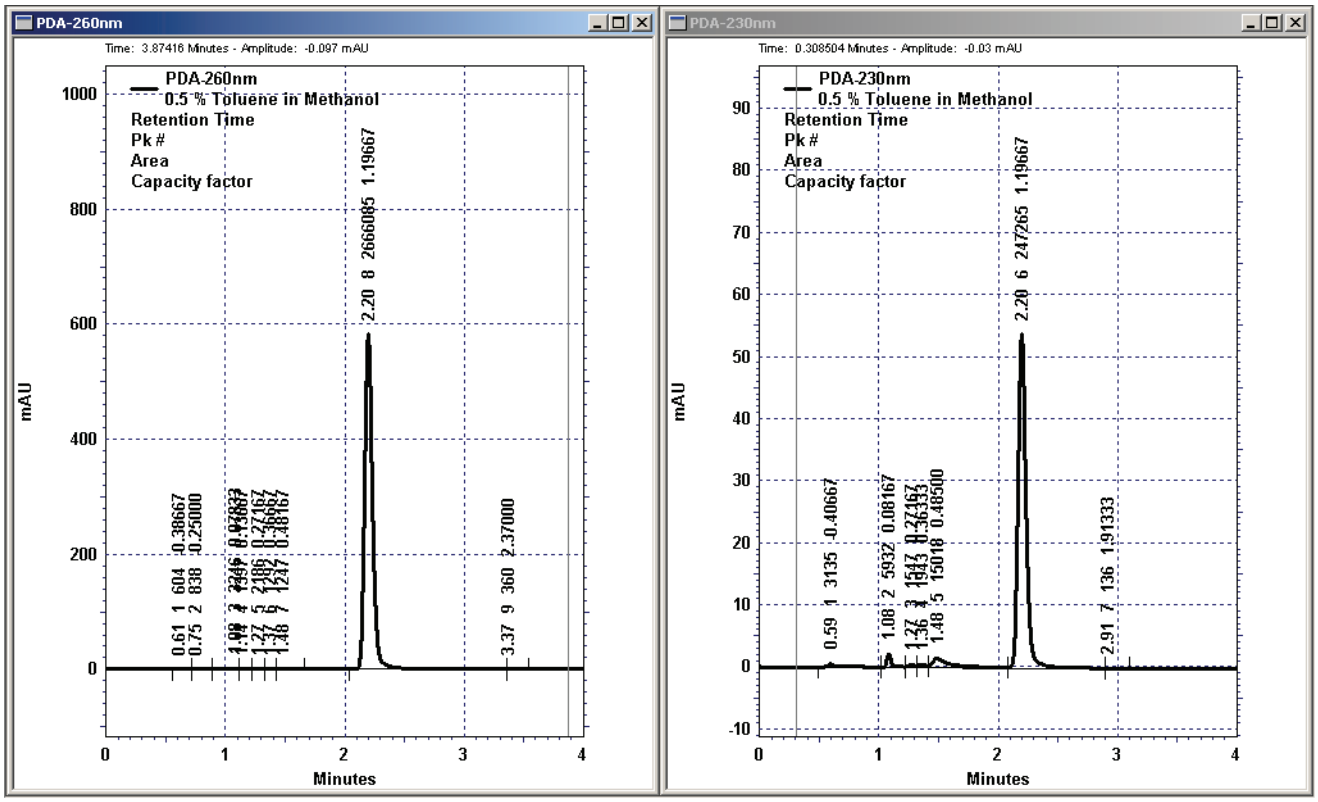

6. Click **Apply To All** to apply these annotations to both the 230 nm and the 260 nm discrete channel chromatograms.

7. Click **OK** to exit the dialog box.

   Both of your chromatograms now contain annotations for Pk#, retention time, area, and capacity factor. By default, the orientation of the annotations is at a 90° angle to the baseline. See Figure 120.

> **Note** If you attempted to add the Name annotation at this point, you will notice that the peaks are not named. To identify the peaks with names, the peaks must be identified in a peak table. You will add a peak table to your method in Chapter 8, "Specifying the Calibration Curve Parameters."

**Figure 120.** Vertically tiled chromatograms with annotations



# Adding Integration Events Graphically

In this tutorial, you learn how to graphically add integration events to a method. In addition, you learn how to graphically add manual integration fixes to individual data files.

A method in ChromQuest contains an Integration Events table for each analysis channel.

Depending on the detector you are using, your method should include the following analysis channels:

- For the Surveyor UV/Vis Plus Detector, which is a dual-wavelength detector, the analysis channels include the wavelengths you entered in the Surveyor UV/Vis page of the Method – Instrument Setup window.
- For the Surveyor PDA Plus Detector, which is a photodiode detector capable of scanning the UV/Vis range from 190 to 800 nm, the analysis channels include the wavelengths you entered in the Surveyor PDA Plus page of the Method – Instrument Setup window and the wavelengths you entered in the Multi-Chromatogram page of the Method – Spectral Options dialog box.
- For the Surveyor FL Plus Detector, which is a programmable, single-wavelength detector, the method does not contain analysis channels.
- For the Surveyor RI Plus Detector, which is a bulk properties detector, the method does not contain analysis channels.

In addition to an Integration Events table associated with the method, the integration of a chromatogram can be affected by a Manual Integration Fixes table that is associated with the data file.

**Contents**

# Opening a Stored Data File

In Chapter 6, "Making Your First Injection," you acquired a data file named Preliminary Run.dat. If you did not perform the tutorial in Chapter 6, you can learn how to add integration events to a method by using one of the stored data files supplied with ChromQuest.

### ❖ To open a stored data file

1. Choose **File > Data > Open** from the menu bar.

   The Open Data File dialog box appears.

2. Click the down arrow next to the Look In box and browse to the appropriate directory:

   - If you performed the tutorial in Chapter 6, "Making Your First Injection," you will find the data file Preliminary Run.dat in the directory:

     Drive:\ChromQuest\Projects\Tutorial\Data.

   - If you did not perform the tutorial in Chapter 6, "Making Your First Injection," use the stored data file Multi Calibration Level 6.dat that is supplied with ChromQuest. You will find these data files in the directory:

     Drive:\ChromQuest\Data.

3. From the list of data files, click a data file to select it.

   **Note** If you do not see your file listed, try changing the Files of Type selection to All Files (*.*) so that all the files in the Data directory are shown including those without file extensions.

   The [.dat] file extension is not automatically added to a data file when it is acquired using the single run acquisition dialog box. The [.dat] file extension must be added to the data file name as described on page.

4. Under Options, select **Original / Acquisition** from the Method list.

5. Click the **Preview** button to see a preview of the chromatogram(s) in the data file. See Figure 121.

6. Click the **Description** button to view a description of the data file. See Figure 122.

7. Click **Open** to open the selected data file.

**Figure 121.** Preview of data file Preliminary Run 001.dat

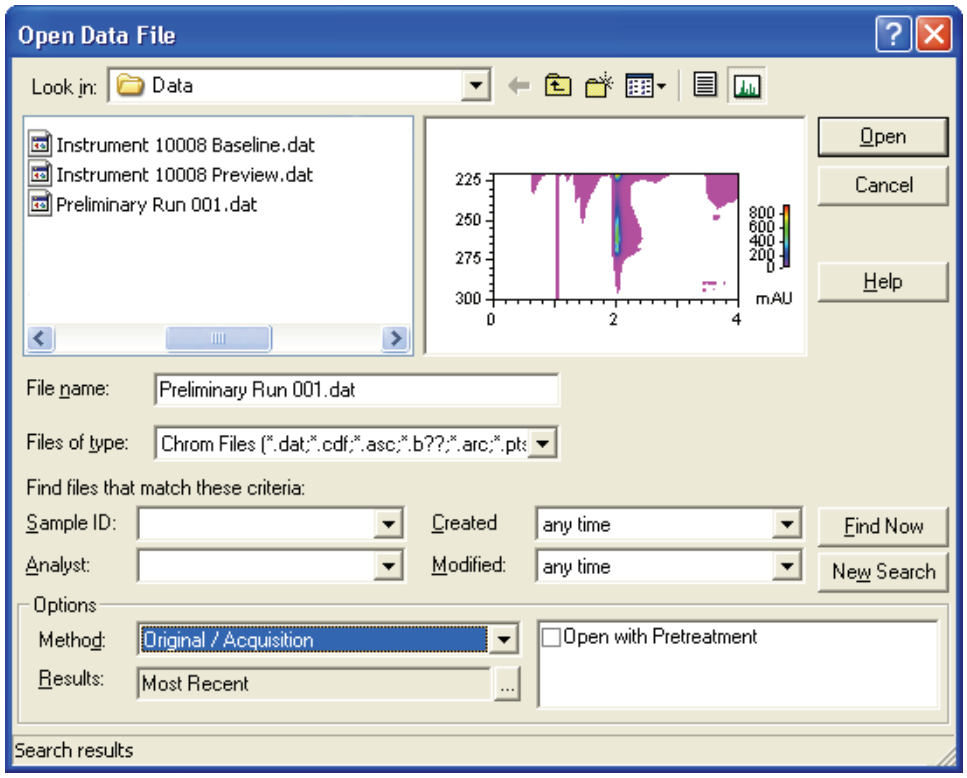
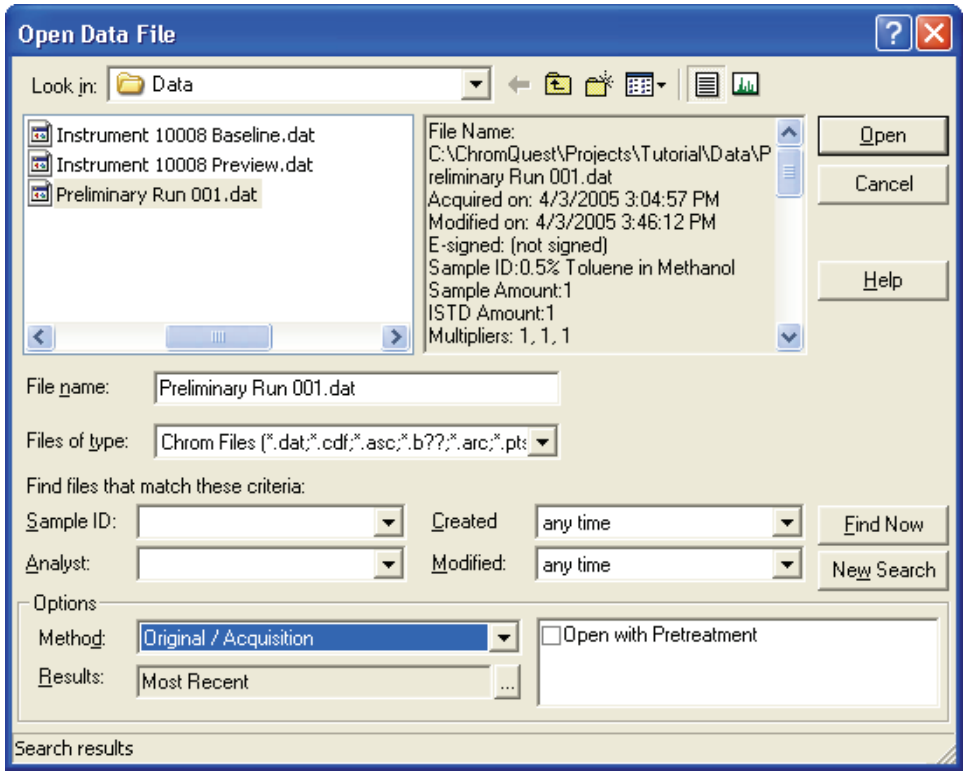


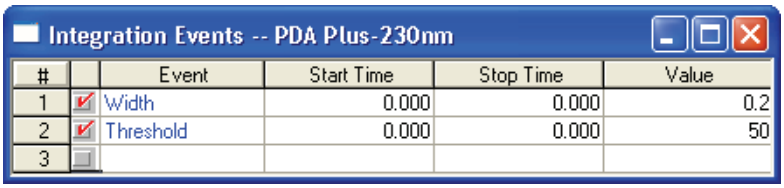

**Figure 122.** Description of data file Preliminary Run.dat



# Adding Integration Events to the Method

By default, the Integration Events table for each wavelength channel contains a Width integration event, set to a value of 0.2 minutes, and a Threshold integration event, set to a value of 50. See Figure 123.

**Figure 123.** Default Integration Events table for the 230 nm wavelength channel




| # | | Event | Start Time | Stop Time | Value |
|---|---|---|---|---|---|
| 1 | ☑ | Width | 0.000 | 0.000 | 0.2 |
| 2 | ☑ | Threshold | 0.000 | 0.000 | 50 |
| 3 | ☐ | | | | |

You can optimize the integration of your chromatograms by adding integration events to their respective Integration Events tables or by changing the default values for the Width and Threshold integration events.

In this tutorial, you will be adding a Threshold integration event to the Integration Events tables for the 230 nm and the 260 nm wavelength channels of your method Test Mix.met.

**Note** You can use one of the stored data files provided with ChromQuest to perform this tutorial. These data files are located in the Drive:\ChromQuest\Data folder. The data files named Multi Level Calibration 1.dat to Multi Level Calibration 6.dat contain a single chromatogram. The data files named LabTest2001.dat to LabTest2007.dat contain a chromatogram for one discrete wavelength, as well as scan data from 220 nm to 360 nm.

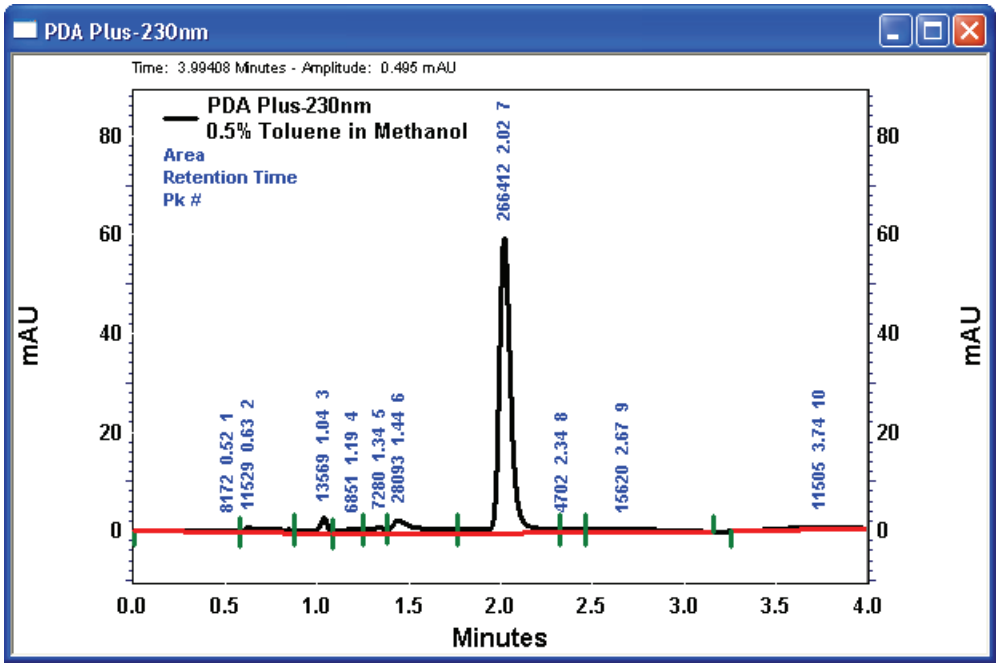
This section contains the following topics:

# Adding Integration Events to the 230 nm Chromatogram

❖ **To add an integration event to the 230 nm chromatogram**

1. If it is not already open, open the Preliminary Run 001.dat data file as described in the previous section: Opening a Stored Data File.
2. If your system has a PDA detector and you collected a scan along with the two discrete wavelength channels, close the Mixed View window.
3. Display the chromatogram for 230 nm. See Figure 124.

   **Figure 124.** Chromatogram for the 230 nm wavelength channel




4. Add a Threshold event to the Integration Event table for the 230 nm wavelength channel:
   a. From the Channel Selector list in the Instrument window toolbar, select the 230 nm wavelength.
   b. Click the chromatogram to activate the integration toolbar at the bottom of the Instrument window. See Figure 125.

**Figure 125.** Integration Events toolbar


| | | |
|---|---|---|
| Width | Minimum Area | |
| Threshold | Turn On Negative Peak | Reset Baseline |
| Shoulder Sensitivity | Disable Peak End | Reset Baseline At Valley |
| Integration Off | Reassign Peak | Adjust Retention Time Window |
| Valley To Valley | Manual Baseline | Adjust Group Range |
| Horizontal Baseline | Manual Peak | Define Single Peak |
| Backward Horizontal Baseline | Split Peak | Define Peaks |
| Lowest Point Horizontal Baseline | Force Peak Start | Define Groups |
| Tangent Skimming | Force Peak Stop | Suggest Sampling Frequency |
| Front Tangent Skimming | Move Baseline | |

c. In the Integration Events toolbar, click the **Threshold** integration event button.

   The message in the Status bar, which is located at the bottom of the Instrument window just below the Integration Events toolbar, instructs you to "*Select Start of Baseline Segment*".

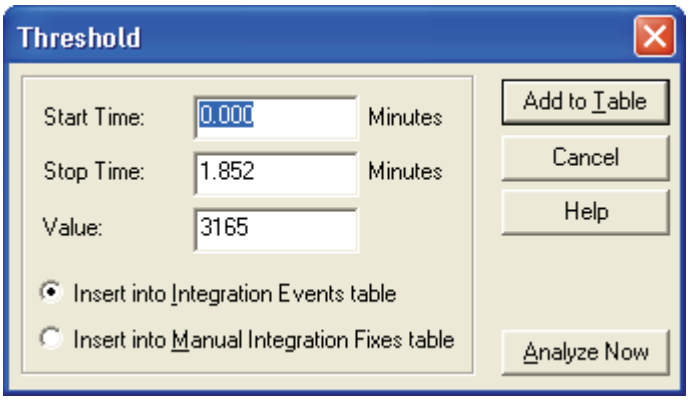
d. Click the baseline at approximately the 0.0 minute time point.

   The Status bar message instructs you to "*Select End of Baseline Segment.*"

e. Click the baseline just in front of the toluene peak, but not including the toluene peak, at approximately the 1.9 minute time point.

   The Threshold dialog box appears. See Figure 126. By default, the Insert Into Integration Events Table option is selected for the Threshold integration event.

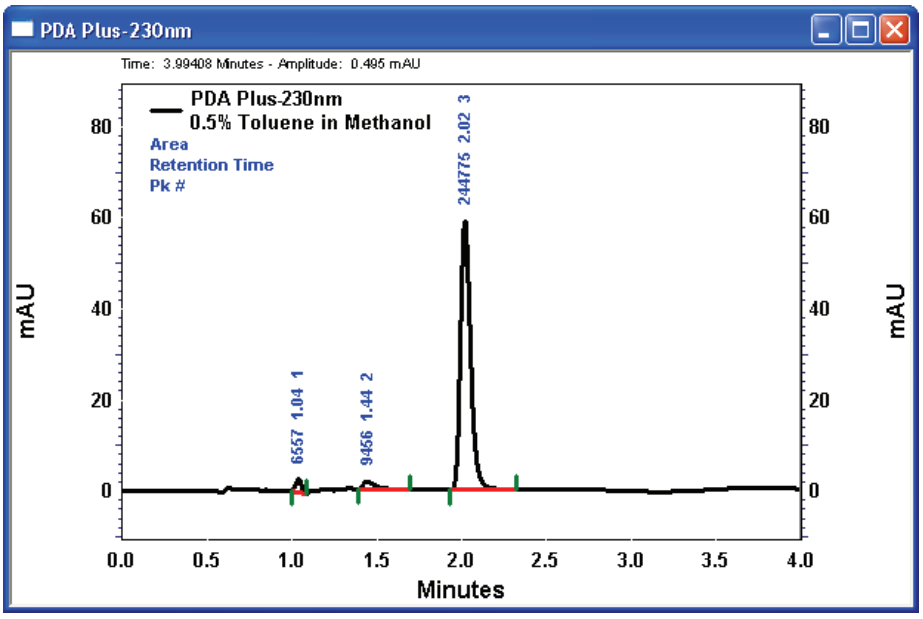
**Figure 126.** threshold dialog box




f. In the Threshold dialog box, click **Analyze Now** (see Figure 126).

This adds the integration event to the integration events table, analyzes the chromatogram, and redraws the baseline. See Figure 127.

**Figure 127.** Chromatogram for 230 nm




5. In the Command toolbar, click the **Integration Events** button to review the Integration Events table for the 230 nm wavelength channel. See Figure 128.

**Figure 128.** Integration Events table for the 230 nm wavelength channel

Integration Events -- PDA Plus-230nm

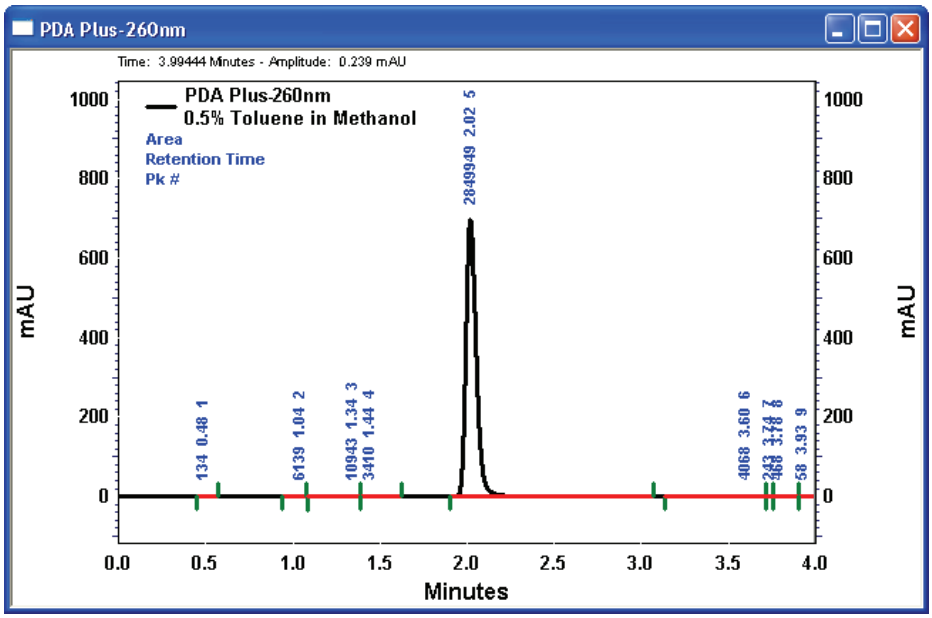
| # | | Event | Start Time | Stop Time | Value |
|---|---|---|---|---|---|
| 1 | ☑ | Width | 0.000 | 0.000 | 0.2 |
| 2 | ☑ | Threshold | 0.000 | 0.000 | 50 |
| 3 | ☑ | Threshold | 0.000 | 1.852 | 3165 |
| 4 | ☑ | | | | |

# Adding Integration Events to the 260 nm Chromatogram

### ❖ To add an integration event to the chromatogram for 260 nm

1. Display the chromatogram for 260 nm. See Figure 129.

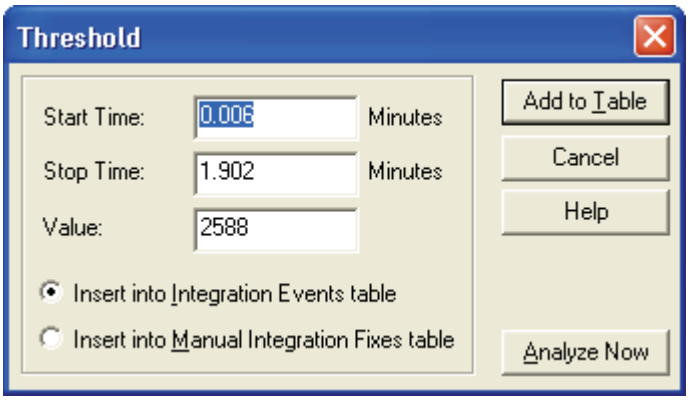
   **Figure 129.** Chromatogram for 260 nm with default integration




2. Add a Threshold event to the Integration Event table for the 260 nm discrete channel:
   a. From the Channel Selector list, select the 260 nm wavelength.
   b. Click the chromatogram to activate the integration toolbar at the bottom of the Instrument window.
   c. In the Integration Events toolbar, click the **Threshold** integration event button.
   d. Click the chromatogram at approximately the 0.0 min time point.
   e. Click the chromatogram at approximately the 1.9 min time point.

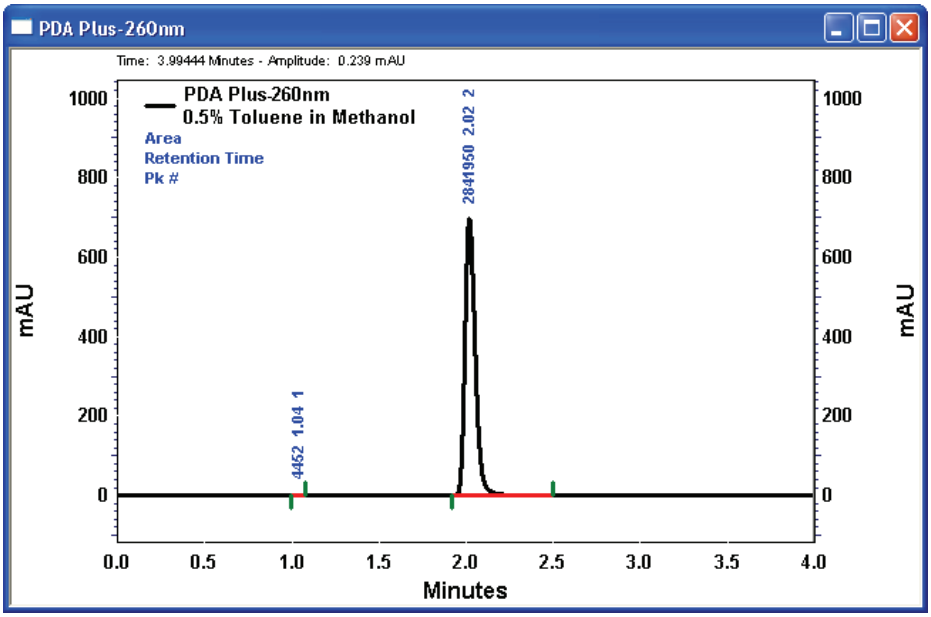
   The Threshold dialog box appears. See Figure 130.

**Figure 130.** Threshold dialog box



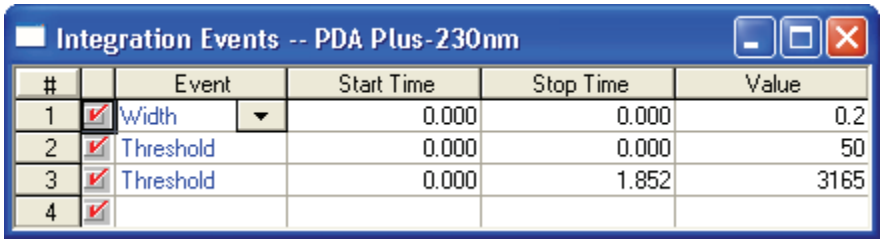

f. In the Threshold dialog box, click **Analyze Now** to add the event to the integration events table and analyze the chromatogram using the event. Figure 131 shows the results of the analysis.

**Figure 131.** Chromatogram window for the 230 nm wavelength channel




3. In the Command toolbar, click the **Integration Events** button to review the Integration Events table for the 230 nm wavelength channel. See Figure 132.

**Figure 132.** Integration events table for the 230 nm wavelength channel

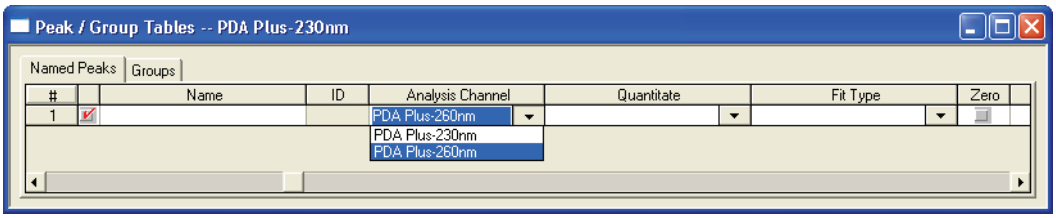


# Specifying the Calibration Curve Parameters

In ChromQuest, the peak table contains the information required to identify the peaks in the chromatograms and the information required to build the calibration curves to quantitate your analytes. In this tutorial, you learn how to add a peak table to your method.

There is one peak table for each detector in the instrument configuration. For multi-wavelength analyses, you use the Analysis Channel column in the peak table to specify the analysis wavelength(s) for the peaks. The wavelength listed in the Title bar is the current selection in the Channel Selection list.

**Figure 133.** Peak table, showing Analysis Channel list for Test Mix.met




With the UV/Vis detector, you can simultaneously collect data on two wavelength channels. With the PDA detector, you can collect scan data from 190 nm to 800 nm, and then quantitate each analyte at its optimal analysis wavelength by adding multi-chromatogram channels to the method.

**Contents**

# Adding a Peak Table Graphically

You can graphically add a peak table to your method using either the Define Peaks dialog box or the Define Single Peak dialog box. Shortcut buttons to these dialog boxes are located in the Integration Events toolbar at the bottom of the Instrument window.

With the Define Single Peak dialog box, you can add define the calibration curve parameters for the peaks in your chromatograms one-by-one. With the Define Peaks dialog box, you can define the calibration curve parameters for a set of peaks.

This section contains the following topics:



## Using the Define Single Peak Dialog Box

In ChromQuest, the peak table contains the information required to identify and quantitate your analytes. In this procedure, you graphically enter the information for a 3-point calibration curve using the data file that you acquired by injecting a solution of toluene (or an analyte of your choice).

### ❖ To create a peak table for your method

1. If it is not already open, open your stored data file, Preliminary Run.dat:
   a. From the Instrument window menu bar, choose **File > Open > Data**.
   b. In the Open Data File dialog box (see Figure 121), browse to the directory where the data file is stored:

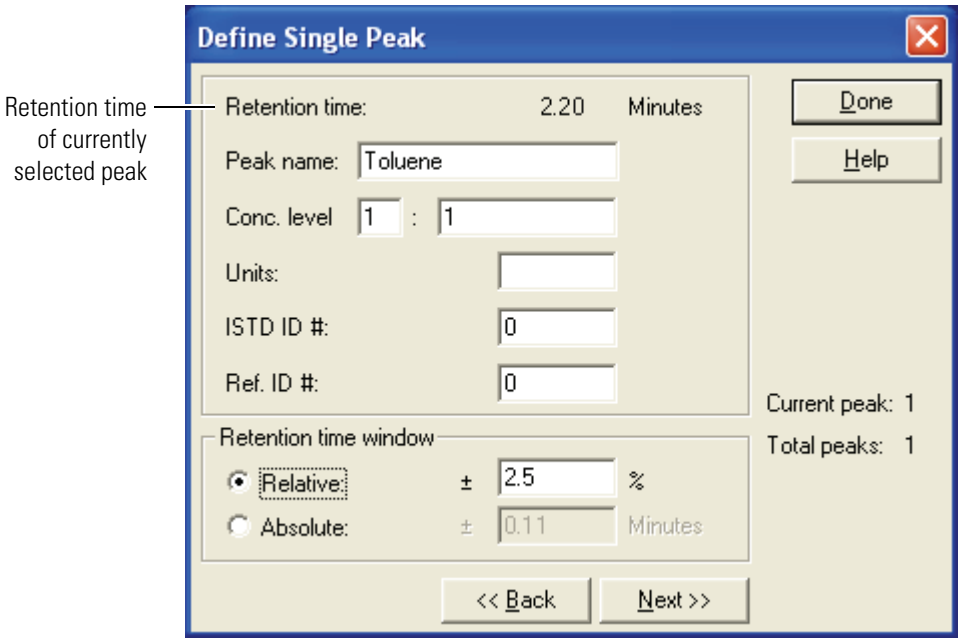
      Drive:\ChromQuest\Projects\Tutorial\Data

   c. Select the data file.
   d. Under Options, select **Results** in the Method list.
   e. Click **Open**.
2. Click anywhere in the Chromatogram window to activate the Integration Events toolbar, and then click the **Define Single Peak**  button.

   The Define Single Peak dialog box appears. See Figure 134.
3. Click the toluene peak in your chromatogram.

   The time listed in the Define Single Peak dialog box should match the retention time of the peak you just selected.

**Figure 134.** Define Single Peak dialog box




4. Type **Toluene** in the Peak Name box.

   In Chapter 10, "Creating a Sequence Table," you create a sequence table that contains a set of calibration standards and a set of unknowns. The calibration levels that you enter in the peak table in steps 5, 6, and 7 are used to create a calibration curve for the quantitation of the unknowns.

   In Chapter 11, "Running and Reprocessing a Sequence," you inject 1, 2, and 3 µL of the toluene solution, instead of preparing a set of calibration standards.

5. To enter the concentration for the first level of the 3-point calibration curve:

   a. Leave the value of 1 in the Conc. Level box to the left of the colon.

   b. Type **1** (or the concentration of your lowest calibration standard) in the box to the right of the colon.

6. To enter the concentration for the second level of the 3-point calibration curve:

   a. Click the toluene peak a second time.

   b. Type **2** (or the concentration of your lowest calibration standard) in the Conc. Level box to the left of the colon.

   c. Type **2** in the box to the right of the colon.

7. To enter the concentration for the third level of a 3-point calibration curve:

   a. Click the toluene peak a third time.

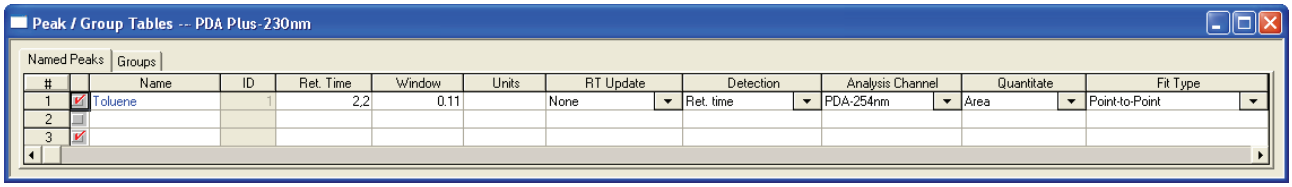
   b. Type **3** (or the concentration of your lowest calibration standard) in the Conc. Level box to the left of the colon.

c. Type **3** in the box to the right of the colon.

> **Note** The concentration values in the Levels columns are unitless. The entry in the Unit column of the peak table is only a label.

8. Leave the other settings in the Define Single Peak dialog box at their defaults.
9. Click **Done**.
10. Click the **Peak/Group Tables** button to review the information in the Peak table.

**Figure 135.** Peak table with entries for the toluene peak

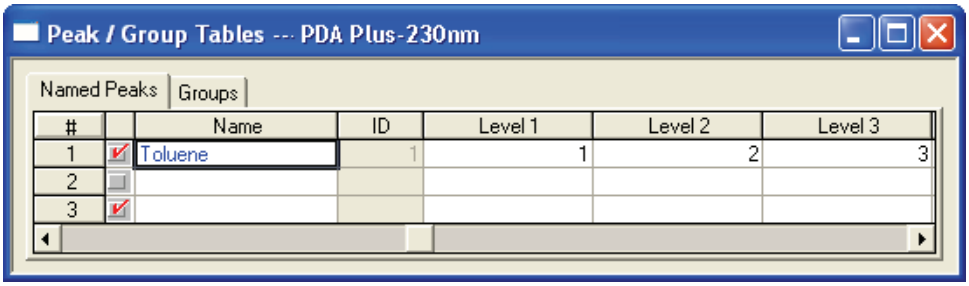



11. Scroll to the right in the peak table until you find the **Fit Type** column. Change the fit type to linear by clicking the down arrow in the Fit Type column and selecting **Linear** from the list.

> **Note** By default, the fit type is point-to-point.

12. Scroll further to the right to review the calibration levels. See Figure 136.

**Figure 136.** Peak table for the PDA Plus detector, showing the calibration levels




Leave the peak table open and proceed to "Modifying the Properties of the Peak Table" on page 164.

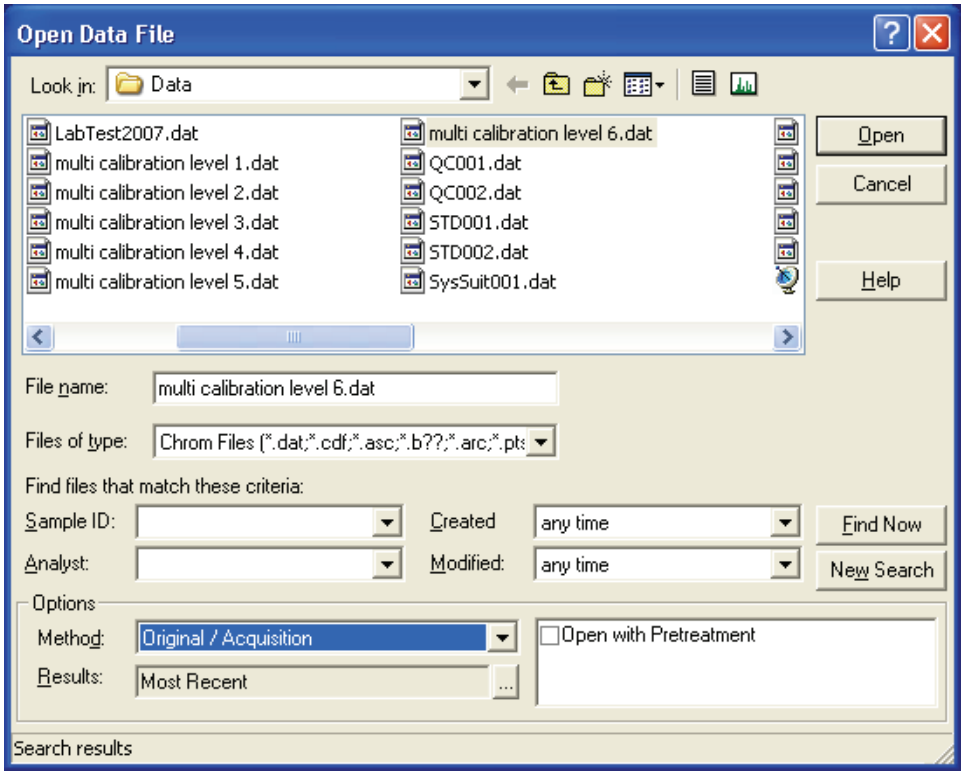
## Using the Define Peaks Dialog Box

If you do not have a stored data file of your own, perform this tutorial to familiarize yourself with the peak table feature. Use the following data file that is supplied with ChromQuest: Multi Calibration Level 6.dat. This data file contains one chromatogram.

### ❖ To create a peak table using the Define Peaks dialog box

1. Open the data file named Multi Calibration Level 6.dat:
   a. From the Instrument window menu bar, choose **File > Open > Data**.
   b. In the Open Data File dialog box, browse to the appropriate directory.
      Drive:\ChromQuest\Data\Projects\Default
   c. Select the data file Multi Calibration Level 6.dat.
   d. Under Options, select **Original / Acquisition** in the Method list.
   e. Click **Open**.

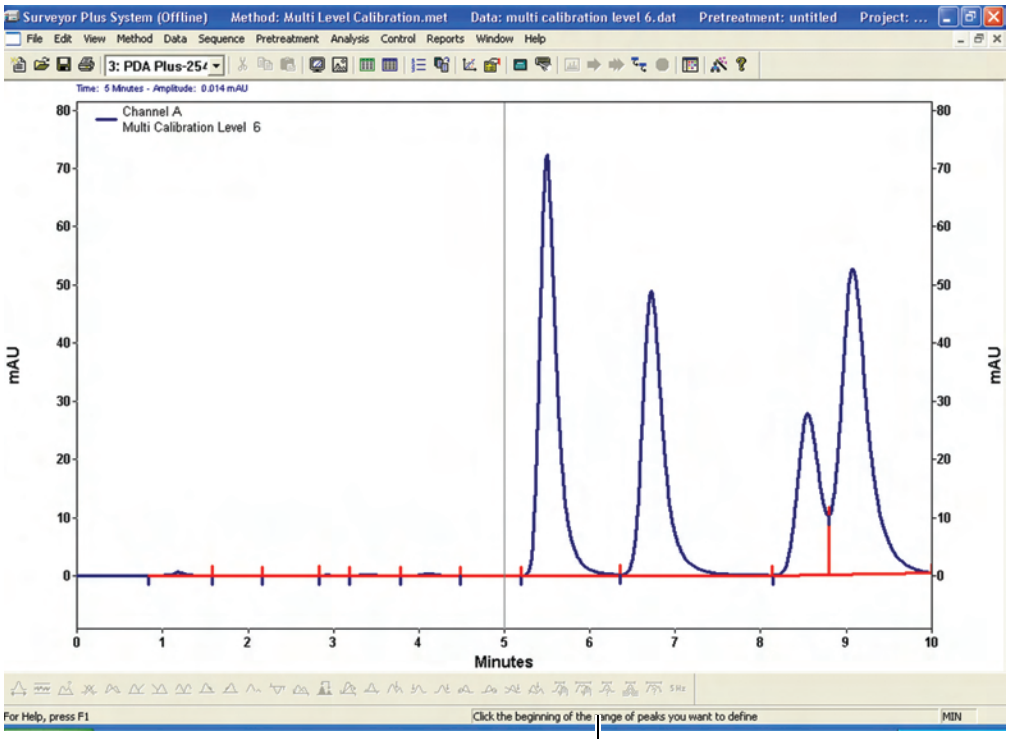
**Figure 137.** Open Data File dialog box, showing the selection of the multi-calibration level 6 data file



2. Click anywhere in the Chromatogram window to activate the Integration Events toolbar, and then click the **Define Peaks** button.

   Instructions appear in the Status bar, which is located below the Integration Events toolbar. The first message instructs you to click the beginning of the range of the peaks you want to define as shown in Figure 138.

**Figure 138.** Instrument window, showing instructions in the status bar



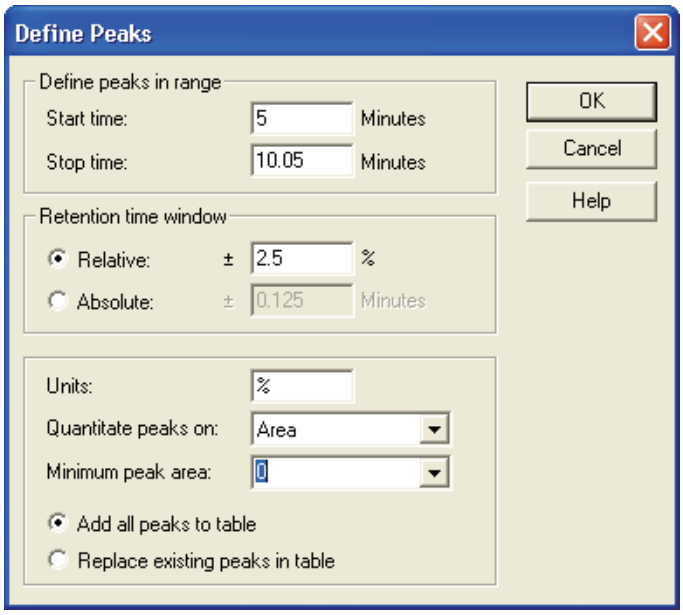

3. Click the chromatogram at approximately the 5 minute time point.

   The message in the Status bar instructs you to click the end of the range of the peaks you want to define.

4. Click the chromatogram at approximately the 10 minute time point.

   The Define Peaks dialog box appears as shown in Figure 139. The Start time box shows the beginning of the selected peak range and the Stop time box shows the end of the selected peak range.

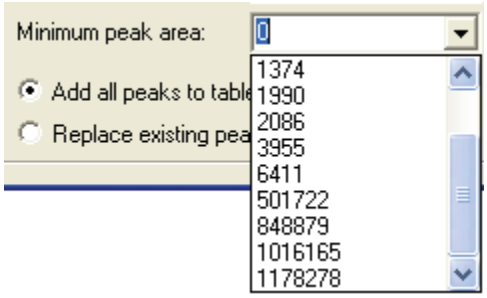
**Figure 139.** Define Peaks dialog box




5. Display the list of integrated peaks by clicking the down arrow for the Minimum Peak Area list as shown in Figure 140.

   Use the listed areas to determine the minimum peak area for your analysis. The peak areas in the list originate from the areas of the integrated peaks in the data file. The last four areas in the table represent the four analyte peaks. The remaining peak areas are due to noise or contaminants.

**Figure 140.** List of integrated peaks in the selected chromatogram




6. Keep all parameters in the Define Peaks dialog box set to the default settings except those that are listed in the following table and are shown in Figure 141.

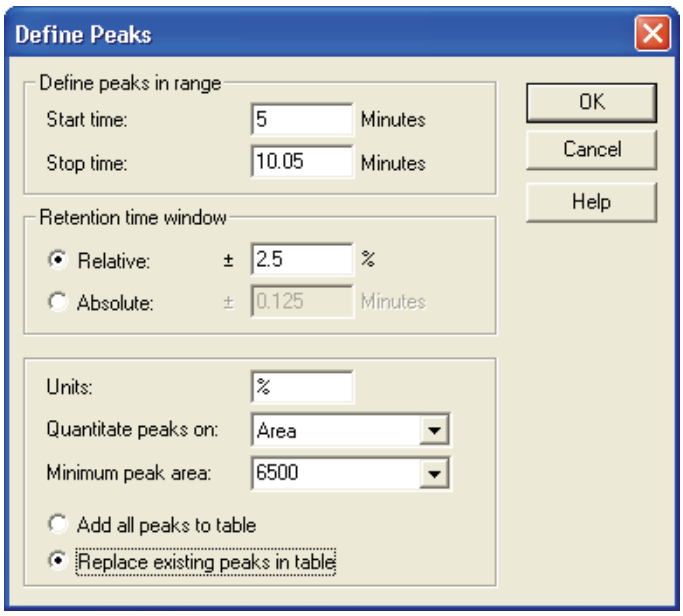
   By default, the retention time window is set to ± 2.5% of the retention time of a named peak and peaks are quantitated based on their areas.

By selecting the Replace existing peaks in table option, you clear any existing entries in the peak table.

| Parameter | Setting | Result |
|---|---|---|
| Minimum Peak Area | 6500* | Specifies that peaks with areas below 6500 area counts are not named in the peak table |
| Replace Existing Peaks In Table | ◉ | Specifies that the existing peak table will be replaced with the current settings |

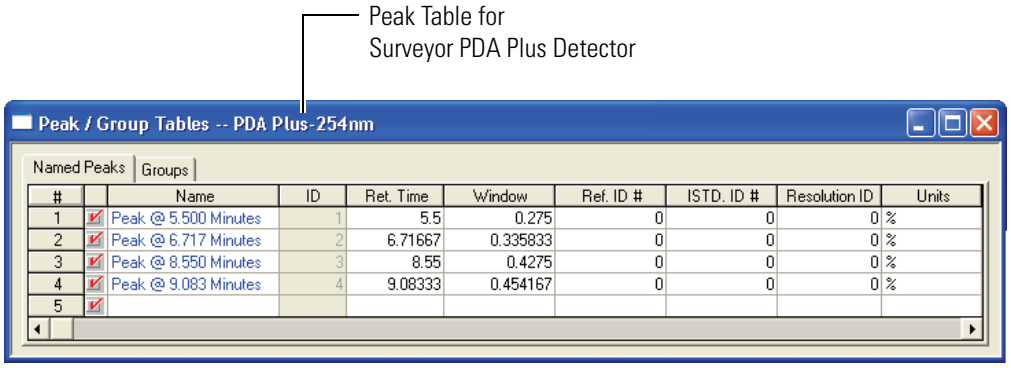
*Note. To enter this setting, type 6500 in the Minimum Peak Area box.

**Figure 141.** Define Peaks dialog box, showing entries




7. Click **OK** to close the dialog box and create the peak table.
8. Click the **Peak/Group Tables** button in the toolbar to review the information in the Peak table. See Figure 142.

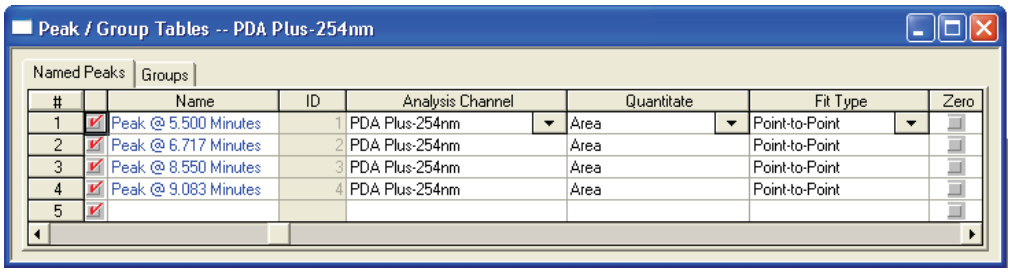
**Figure 142.** New Peak table for Multi Level Calibration.met




9. Scroll to the right in the peak table. Notice that by default the fit type of the calibration curve is set to point-to-point. See Figure 143.

**Note** The Multi Calibration Level data files contain only one chromatogram. For multi-wavelength analyses, select the analysis channel in the Analysis Channel list. Unlike the Integration Events tables, the wavelength listed in the title bar of the Peak / Group Tables window has no real meaning.

**Figure 143.** View of the peak table after scrolling to the right



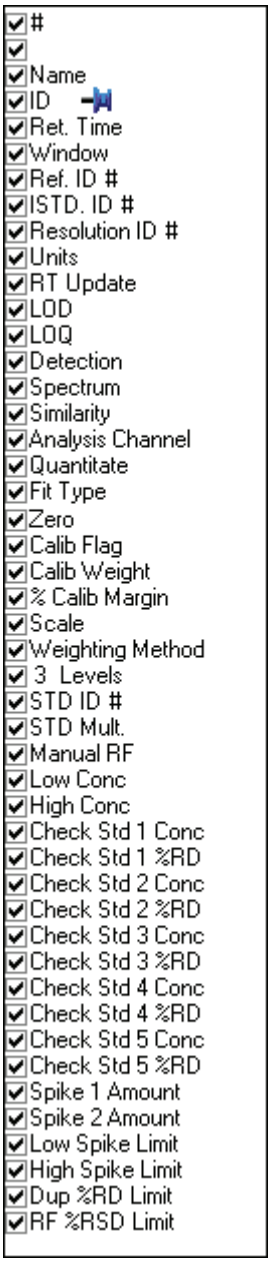

10. Save the method by choosing **File > Method > Save**.

# Modifying the Properties of the Peak Table

By default the peak table contains all the columns shown in Figure 144. You can reduce the number of columns in the table to those that you actually use by clearing the check boxes next to the columns that you do not use.

Because the column selections in the peak table are saved on a per-instrument and per-user basis, you can set the peak table parameters for each instrument controlled from the ChromQuest data system.

**Figure 144.** List of default columns in the peak table



### ❖ To modify the properties of the peak table

1. Right-click in the Peak Tables window and choose **Properties** from the shortcut menu.
2. To avoid excessive scrolling, clear the check boxes next to the columns that will not be used in this tutorial. Keep the columns listed in the following table.

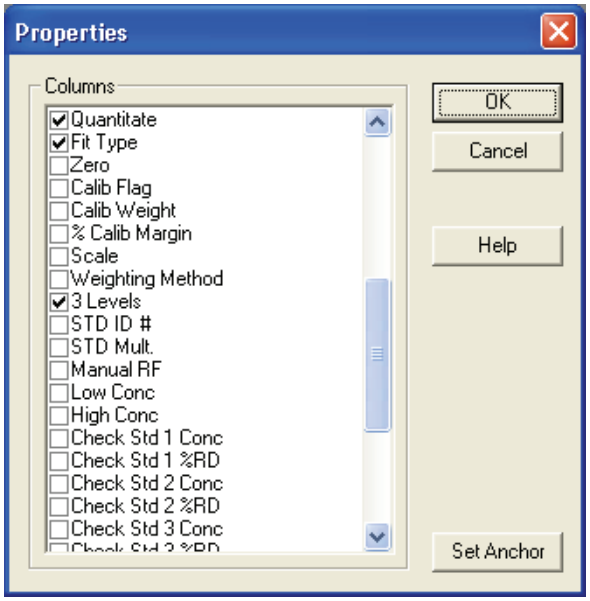
   - #
   - Selection check box
   - Name
   - ID
   - Ret Time
   - Window
   - Detection
   - Analysis Channel
   - Quantitate
   - Fit Type
   - Calib Flag
   - Levels

3. Change the number of calibration levels displayed in the peak table:
   a. In the Properties dialog box, double-click **Levels** to open the Max # of levels box.

   b. Highlight the current value of 10.
   c. Type **3** in the box, and then press ENTER.
   d. Verify that the Levels check box is still selected. See Figure 145.
   e. Click **OK**.

**Figure 145.** Properties dialog box, showing the number of calibration levels displayed in the peak table




4. Save the method by choosing **File > Method > Save**.

# Performing Multi-Wavelength Analyses

The ability to identify and quantitate a peak on more than one analysis channel is a useful feature, especially if your chromatographic peaks are impure or if the optimal analysis wavelengths of the analytes in your sample differ significantly.

This section contains the following topics:

- Modifying the Analysis Channels in the Peak Table
- Modifying the Annotations Listed on the Screen

## Modifying the Analysis Channels in the Peak Table

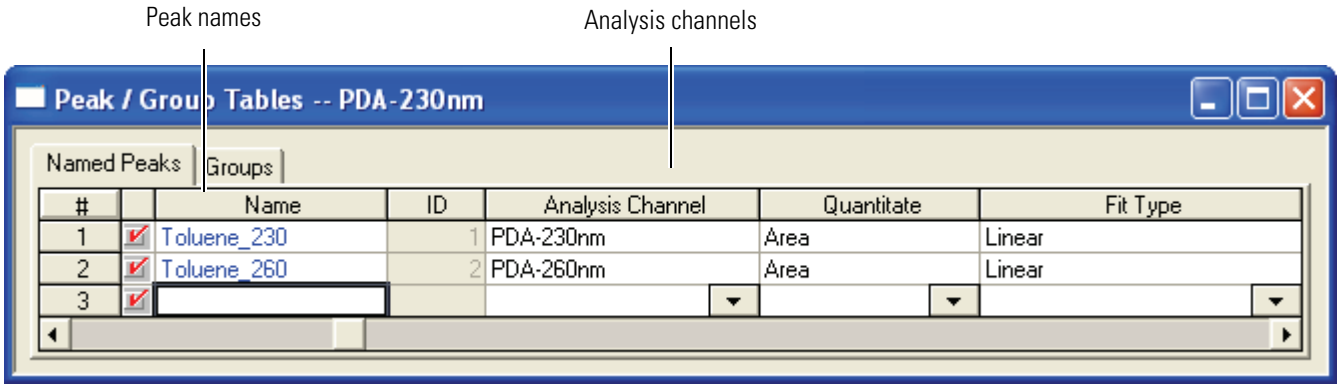
❖ **To identify and quantitate the toluene peak on two Analysis Channels**

1. Verify that the Analysis Channel in the first row is the 230 nm wavelength.
2. Copy the information in row 1 to row 2:
   a. In row 1 of the peak table, click the # column to select the entire row.
   b. Press CTRL+C to copy the information in row 1.
   c. In row 2 of the peak table, click the # column to select the entire row.
   d. Press CTRL+V to paste the information in row 1.

3. In the Analysis Channel column, select the 260 nm wavelength from the list of available wavelengths.
4. Right-click in the peak table and choose **Renumber Peak IDs** from the shortcut menu.
5. Change the name of the peak on the first row of the peak table to **Toluene_230nm**.
6. Change the name of the peak on the second row of the peak table to **Toluene_260nm**.

   Give the two toluene peaks unique names to help you identify their respective calibration curves when you review the calibration information. See Figure 146.
7. Save the method by choosing **File > Method > Save**.

**Figure 146.** Peak table for Test Mix.met




## Modifying the Annotations Listed on the Screen

To view the names of the peaks on your view screen, add the Name annotation.

### ❖ To add the Name annotation to the chromatograms

1. Click a chromatogram on your view screen to open the Chromatogram shortcut menu.
2. In the shortcut menu, choose **Annotations** to open the Trace Annotation Properties dialog box. See Figure 119.
3. In the Available Annotations box, double-click the **Name** annotation to add it to the Show The Following Annotations box.
4. Click **Apply To All**.
5. Click **OK** to exit the Trace Annotation Properties dialog box.
6. Save the method by choosing **File > Method > Save**.
7. In the Command toolbar, click the **Analyze** button.

   The peak in the chromatogram for the 230 nm wavelength channel is named Toluene_230nm. The peak in the chromatogram for the 260 nm wavelength channel is named Toluene_260nm.

# Adding a Custom Report to the Method

In this tutorial, you learn how to add one of the standard method custom report templates to your method and how to create your own method custom report template that can be stored in a template folder and then added later to a method.

To print an individual report for each data file, you must add a method custom report to your method. You can add one of the six standard custom templates, or you can create your own method custom report template. After you create and save a method custom report template, you can add it to other methods, as well as the current method.

**Contents**



## Adding a Standard Custom Report to the Method

By default, the Custom Report section of the method is blank until you add a report template.

❖ **To add a Custom Report to your method**

1. Open the Offline Instrument window for your instrument.
2. Ensure that the method you created while performing the tutorial in Chapter 4, "Creating Methods," is open.

   The name of the active method is listed in the title bar of the Instrument window.
3. Click the **Edit Custom Report**  button.

   If this is a new method, a blank page appears.
4. From the Instrument window menu bar, choose **File > Report Template > Open** to open the Open Report Template File dialog box and access one of the six standard custom report templates:
   - External Standard-horiz.rep
   - External Standard-Vertical.rep
   - External Standard.srp

- Internal Standard.srp
- Area%.srp
- Normalization.srp

5. Select the **External Standard.srp** custom report template. See Figure 147.

   Templates with the file extension .srp can be automatically viewed by choosing **Reports > View,** and then making a selection from the menu shown in Figure 148.

   The External Standard.srp custom report template appears on your screen. The contents of the active data file, Preliminary Run 001.dat are displayed. Your custom report page will look similar to the one shown in Figure 149.

6. Choose **File > Method > Save** to save the method.

**Figure 147.** Open Report Template dialog box

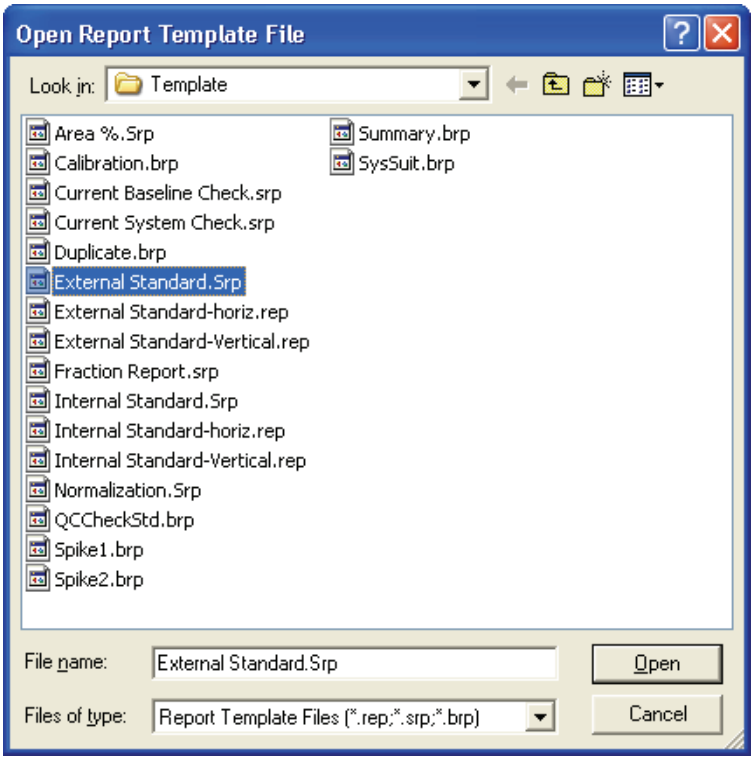


**Figure 148.** Selecting the report that you want to view on the view screen

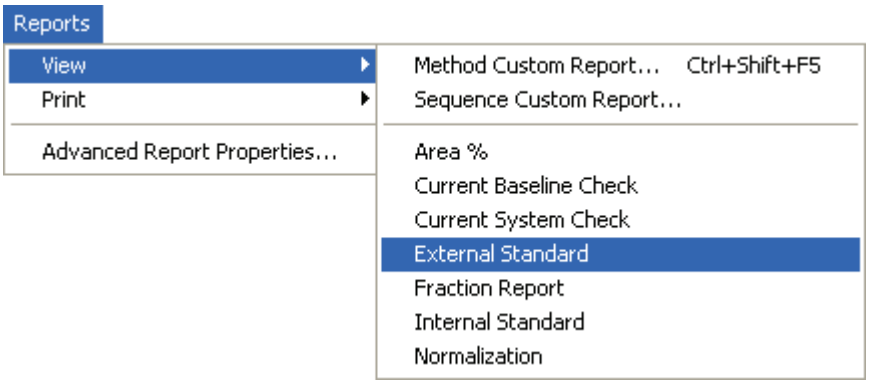
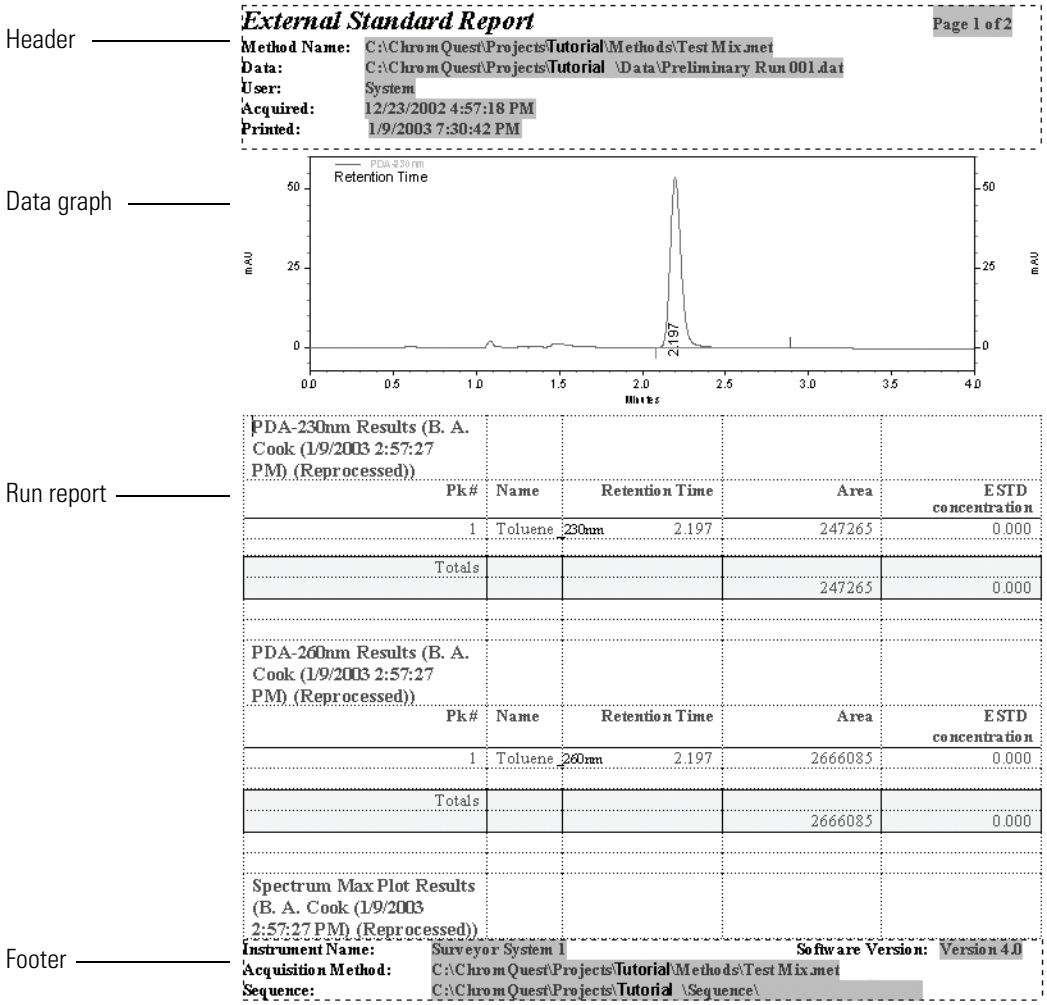



**Figure 149.** Method Custom Report created using the *External Standard.srp* template



# Creating a Custom Report Template

To suit your reporting requirements, you can modify a custom report template or you can create a completely new custom report template.

If it is not already open, click the **Edit Custom Report** button to open the Custom Report page of the method. Ensure that the report page contains the ESTD Standard.srp custom report template that you added while performing the procedure "Adding a Standard Custom Report to the Method" on page 169.

Create your own custom report template by modifying the ESTD Standard.rep custom report template by performing the following procedures in this section:

- Adding Text to a Custom Report Template
- Modifying Data Graph Annotations
- Changing the Appearance of the Data Graph
- Modifying the Run Report

## Adding Text to a Custom Report Template

❖ **To add the name of your company to the top of the report**

1. Place the cursor between the report header and the data graph of the chromatogram (see Figure 149). If the header is not visible, right-click the report and choose **Header / Footer** from the shortcut menu.
2. Type the name of your company, for example, **Quality Performance Laboratories**.
3. Using the Custom Report toolbar (see Figure 150), change the font type for the company name to arial, 14 point, bold, and then center justify the heading:
   a. Highlight the company name.
   b. Select **Arial** from the Font Name combo box.
   c. Select **14** from the Font Size combo box.
   d. Click the **Bold** (B) button.
   e. Click the **Center Justify** button to horizontally center the company name at the top of the report page.

**Figure 150.** Custom Report toolbar

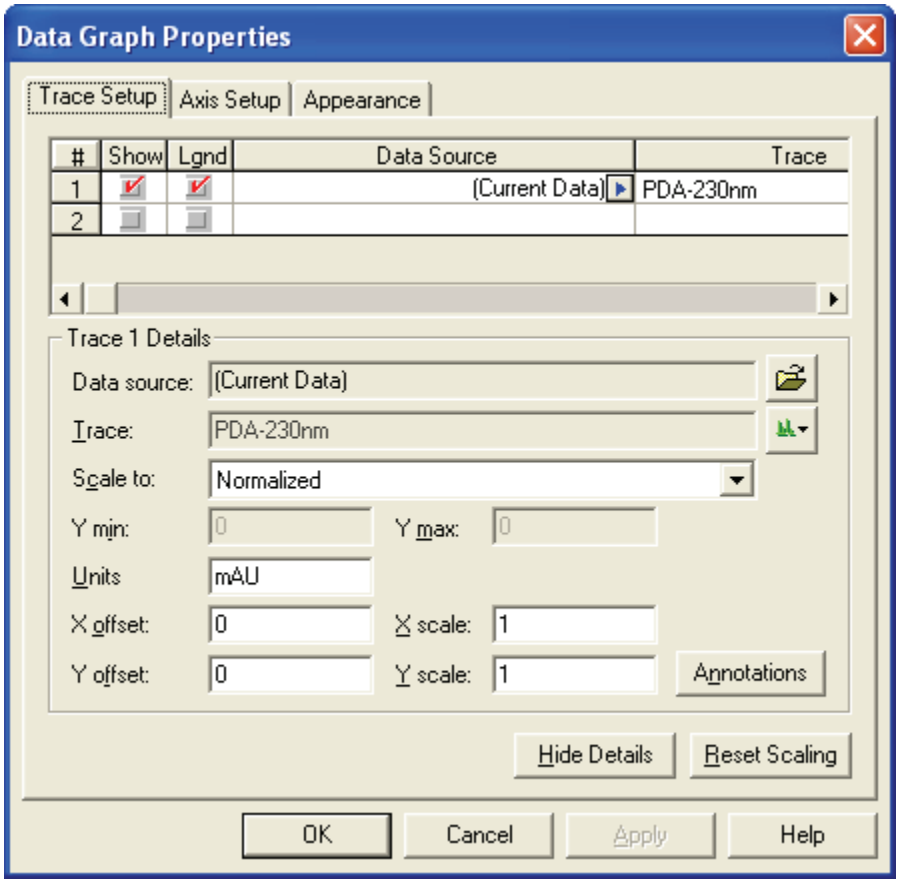
# Modifying Data Graph Annotations

By default, the template contains one data graph. The retention time of the peaks to 3 decimal places is its only annotation.

### ❖ To modify the annotations on the data graph

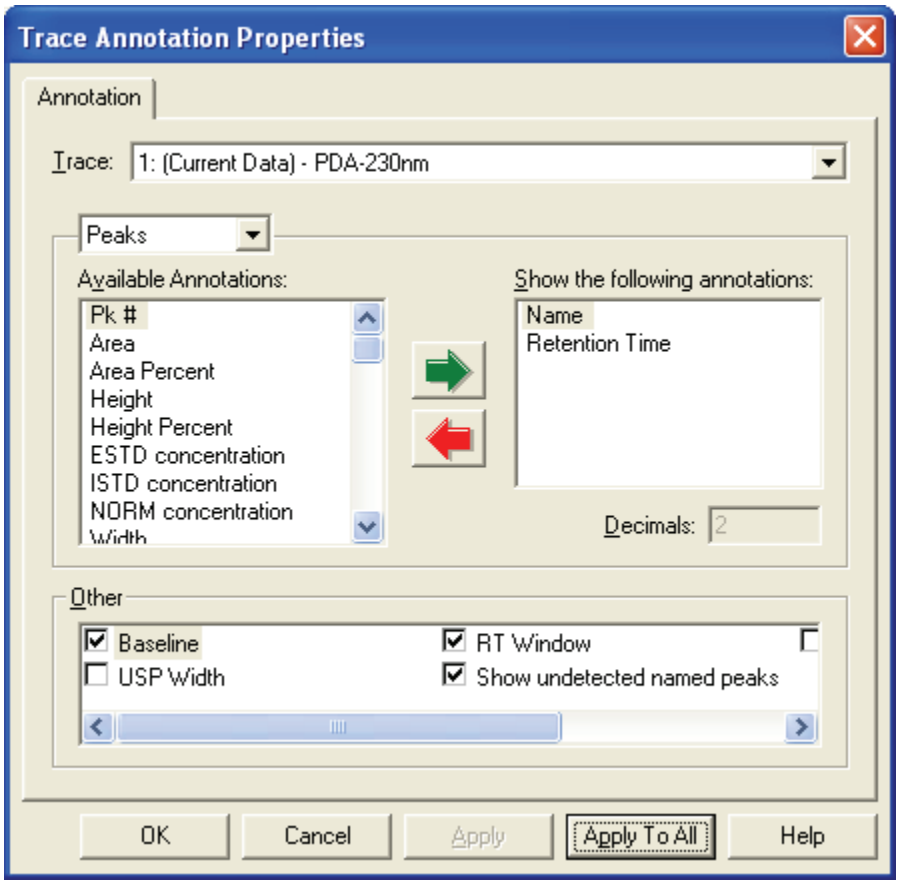
1. Double-click the data graph to open the Data Graph Properties dialog box.
2. Click the **Trace Setup** tab to open the Trace Setup page shown in Figure 151.

   **Figure 151.** Trace Setup page of the Data Graph Properties dialog box




3. In the Trace Setup page, click **Annotations** to open the Trace Annotation Properties dialog box shown in Figure 152.

**Figure 152.** Trace Annotation Properties dialog box




4. In the Trace Annotation Properties dialog box, do the following:
   a. In the Available Annotations list, double-click the **Name** and **Retention Time** annotations.

      The Name and Retention Time annotations appear in the Show the following annotations list.

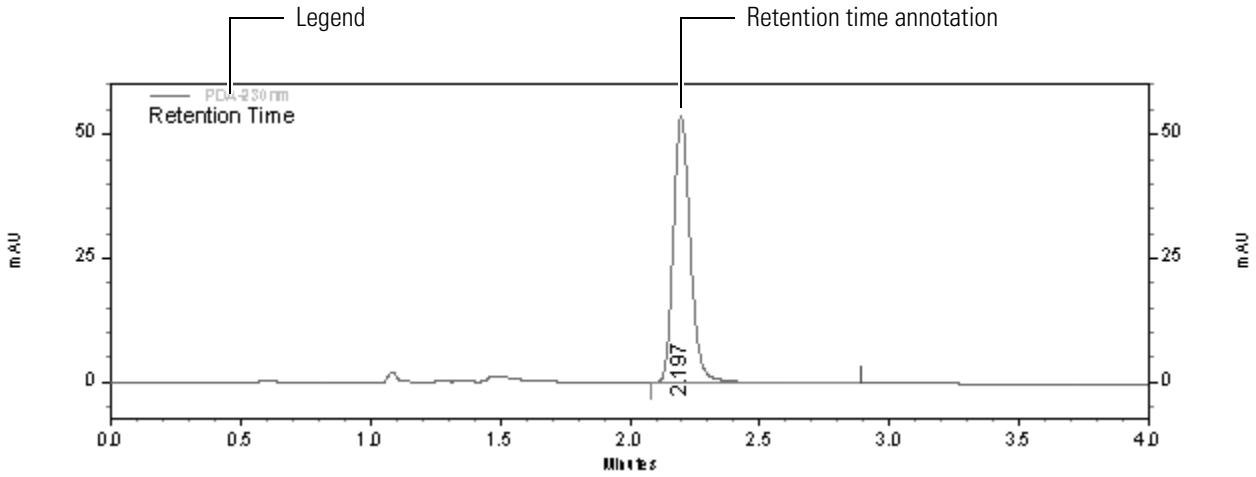
   b. Change the number of decimal places for Retention Time to 2:
      i. Click the **Retention Time** annotation in the Show the following annotations list.
      ii. Highlight the number 3 in the Decimals box. Then, type **2.**
   c. Under **Other**, select the RT Window check box.
   d. Click **Apply To All**.
   e. Click **OK** to exit the Trace Annotation Properties dialog box.

Keep the Data Graph Properties dialog box open and go to the next topic, "Changing the Appearance of the Data Graph."

## Changing the Appearance of the Data Graph

By default, the font size of the data graph legend is 6 point and its color is gray. By default, annotations are listed at a 90° angle. See Figure 153.

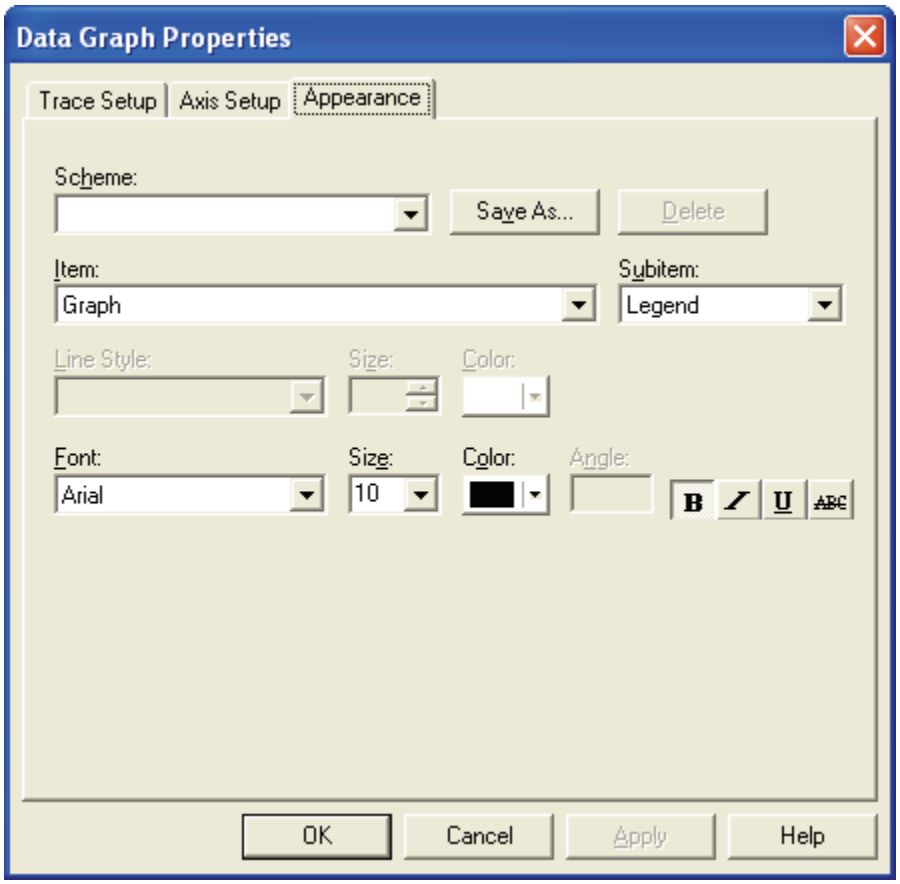
**Figure 153.** Data graph of the 230 nm chromatogram of toluene




### ❖ To change the appearance of the data graph

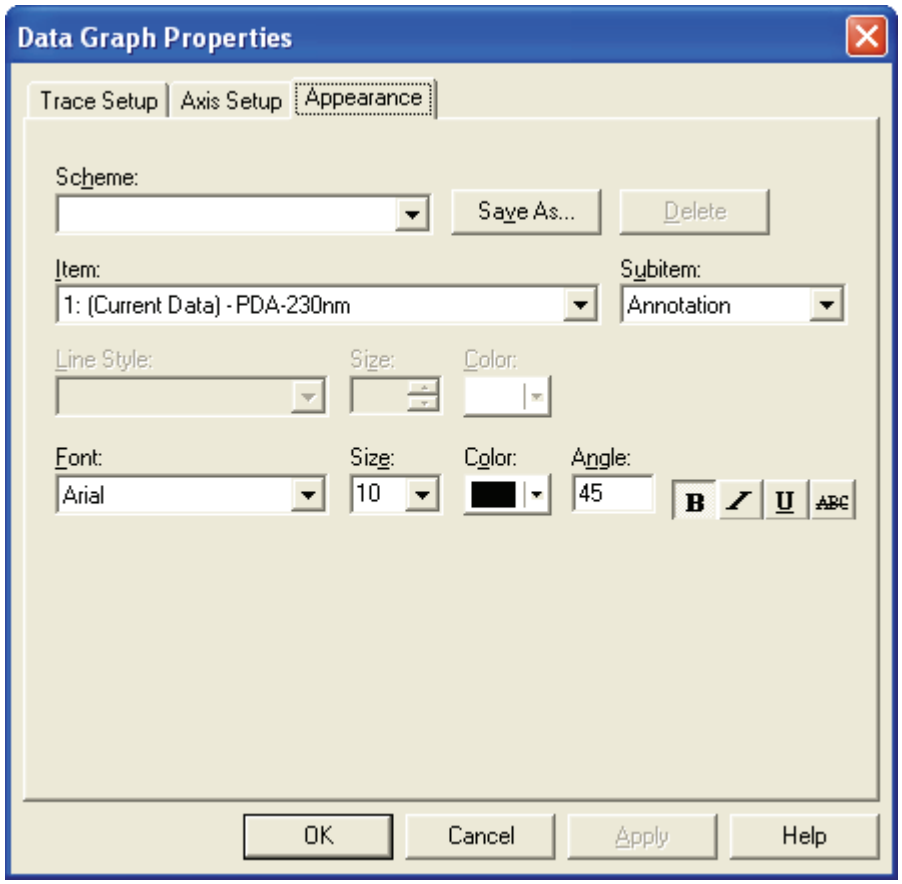
1. In the Data Graph Properties dialog box, click the **Appearance** tab to open the Appearance page shown in Figure 154.
2. Change the appearance of the legend, which is located in the top left corner of the data graph:
   a. From the Item list, select **Graph**.
   b. From the SubItem list, select **Legend**.
   c. From the Size list, select **10**.
   d. From the Color list, select the black square.
   e. Click **Apply**.

**Figure 154.** Appearance page of the Data Graph Properties dialog box




3. Change the appearance of the trace Annotations:
   a. From the Item list, select the trace. For example, the trace for the Surveyor PDA will be named, 1:(Current Data)-PDA-230 nm. See Figure 155.
   b. From the Font list, select **Arial**.
   c. From the Size list, select **10**.
   d. From the Angle list, select **45**.
   e. Click the **Bold** button.
   f. Click **Apply**.

**Figure 155.** Appearance Properties dialog box

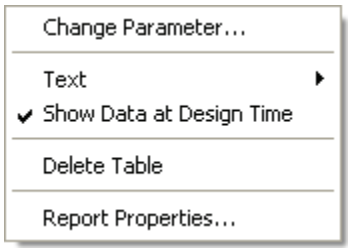



## Modifying the Run Report

### ❖ To modify the Run Report

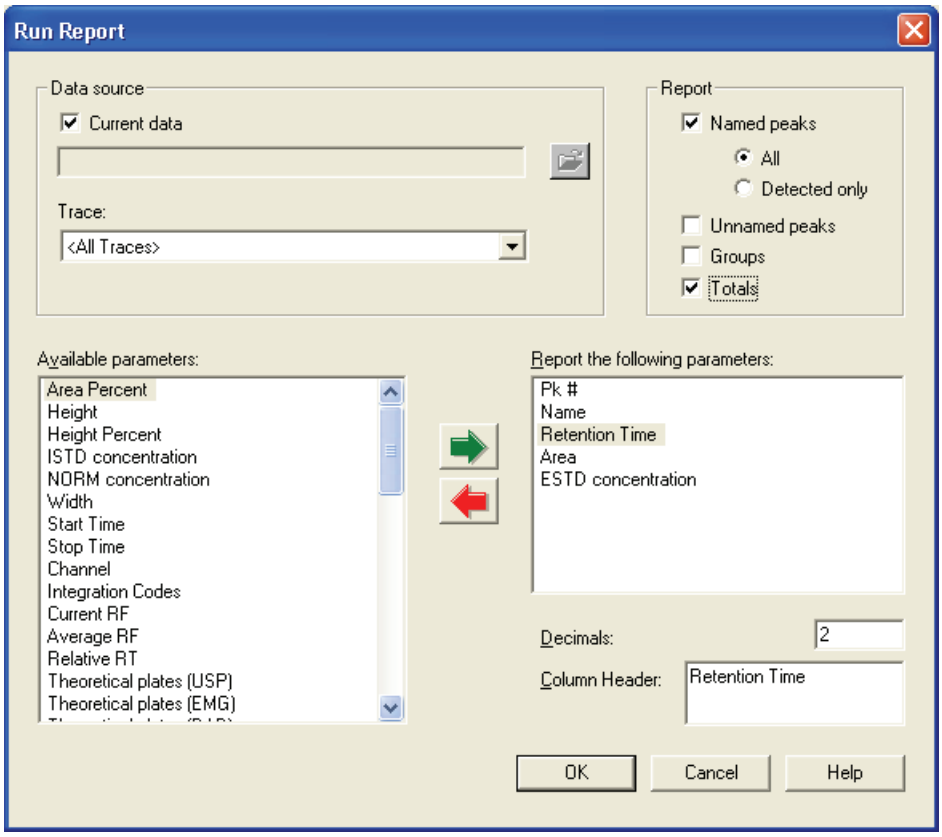
1. Click the Run Report section of the Custom Report template. Then right-click the Run Report section (see Figure 149) to open a shortcut menu. See Figure 156.
2. Verify that the menu item **Show Data At Design Time** is checked.

   **Figure 156.** Run Report shortcut menu

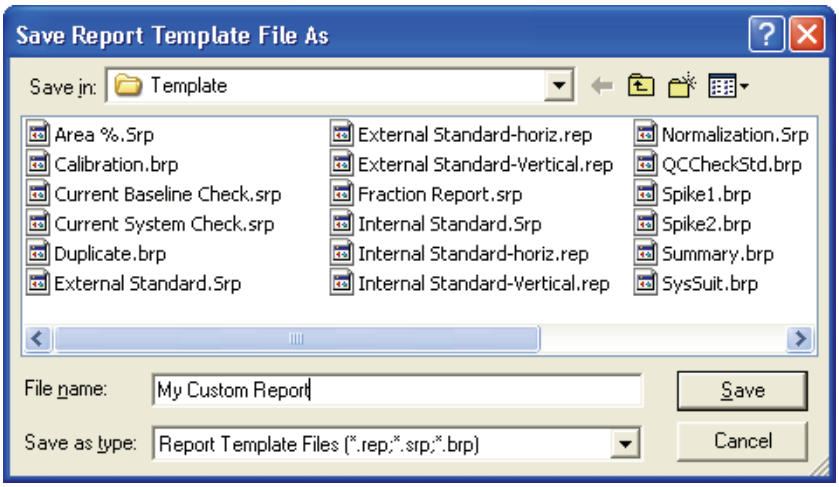
3. In the shortcut menu, choose **Report Properties** to open the Run Report dialog box. See Figure 157.

**Figure 157.** Run Report dialog box




4. In the Run Report dialog box, do the following:
   a. In the Report the following parameters box, click **Retention Time** to select it.
   b. Change the number of decimal places to 2 by highlighting the number 3, and then typing **2** in the Decimals box.
   c. In the Report the following parameters box, click **ESTD Concentration** to select it.
   d. Change the number of decimal places to 2 by highlighting the number 3, and then typing **2** in the Decimals box.
   e. Click **OK** to exit the Run Report dialog box.
5. Save the new custom report template in the Templates folder of the Tutorial project:
   a. Choose **File > Report Template > Save As** from the menu bar to display the Save Report Template As dialog box. See Figure 158.

**Figure 158.** Save Report Template As dialog box




b. Browse to the appropriate directory.

   Drive:\ChromQuest\Projects\Tutorial\Template

c. In the Filename box, type in the name **My Custom Report Template** for the Custom Report Template.

d. Click **Save**.

   ChromQuest adds the *.rep* extension to the file.

> **Note** If you want the template to appear in the list that you access by choosing **Reports > View** from the menu bar, add an [.srp] extension.

6. Save the method, Test Mix.met, by choosing **File > Method > Save.**

The custom report produced using your new template will look similar to the one shown in Figure 159.

**Figure 159.** Modified method custom report

Header

Data graph

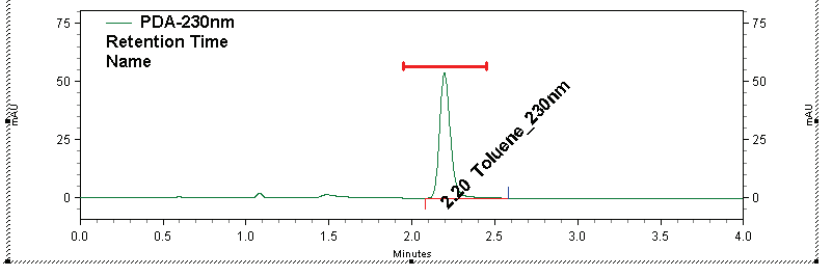
Run report

Footer

*External Standard Report* Page 1 of 2

Method Name: C:\ChromQuest\Projects\Tutorial\Method\Test Mix.met
Data: C:\ChromQuest\Projects\Tutorial\Data\Preliminary Run 001.dat
User: System
Acquired: 12/23/2002 4:57:18 PM
Printed: 1/22/2003 2:56:55 PM

Quality Performance Laboratories




**PDA-230nm Results (System (1/22/2003 2:56:54 PM) (Reprocessed))**

| Pk # | Name | Retention Time | Area | ESTD concentration |
|---|---|---|---|---|
| 1 | Toluene_230nm | 2.20 | 244561 | 2.15 |
| Totals | | | 244561 | 2.15 |

**PDA-260nm Results (System (1/22/2003 2:56:54 PM) (Reprocessed))**

| Pk # | Name | Retention Time | Area | ESTD concentration |
|---|---|---|---|---|
| 8 | Toluene_260nm | 2.20 | 2666085 | 2.14 |
| Totals | | | 2666085 | 2.14 |

Instrument Name: Surveyor System 1 Software Version: Version 4.0
Acquisition Method: C:\ChromQuest\Projects\Tutorial\Method\Test Mix.met
Sequence: C:\ChromQuest\Projects\Tutorial\Sequence\

# Creating a Sequence Table

In this tutorial, you learn how to use the Sequence Wizard to create a basic acquisition sequence table. In addition, you learn how to modify this basic sequence table to suit your applications.

The sequence table contains the information that is required to inject a set of samples or to reprocess a set of stored data files. ChromQuest contains a Sequence Wizard that guides you through the creation of a sequence table.

**Contents**



## Using the Sequence Wizard to Create a Sequence Table

In this tutorial you use the Sequence Wizard to create an acquisition sequence that contains three calibration standards and three unknowns. For convenience, you make all the injections from one vial.

### ❖ To use the Sequence Wizard to create a sequence table

1. From the Instrument window, choose **File > Sequence > Sequence Wizard** to start the Sequence Wizard.

   The Sequence Wizard opens to the Method page.

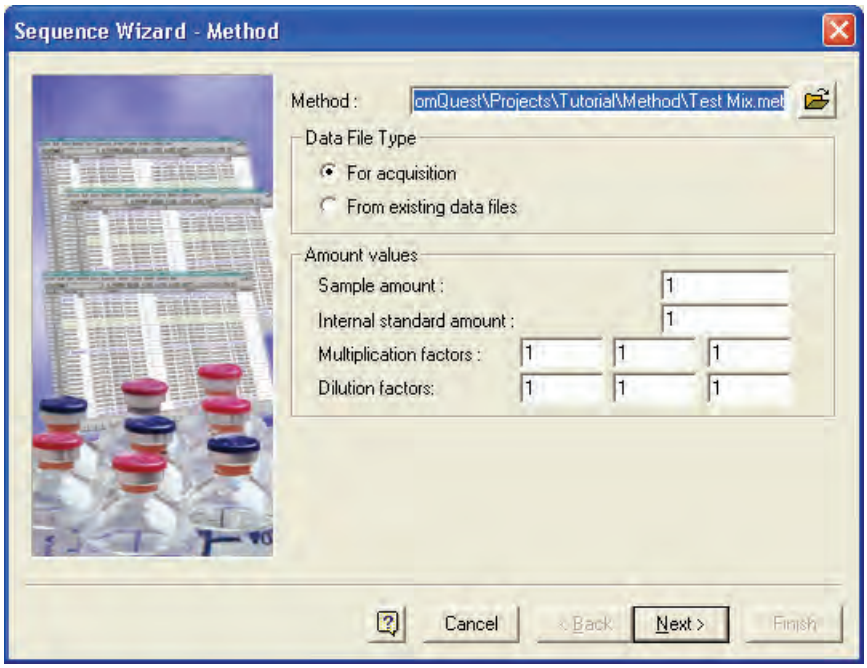
2. In the Method page, do the following:

   a. Except for the method, keep the settings in the Method page at their defaults, as shown in Figure 160. If Test Mix.met is not listed in the Method list, browse to Test Mix.met or an appropriate alternative method.

   b. Click **Next** to open the Unknowns page of the Sequence Wizard.

> **Note** When you open the Sequence Wizard, the Method list displays the active method listed in the title bar of the Instrument window.

**Figure 160.** Method page of the Sequence Wizard

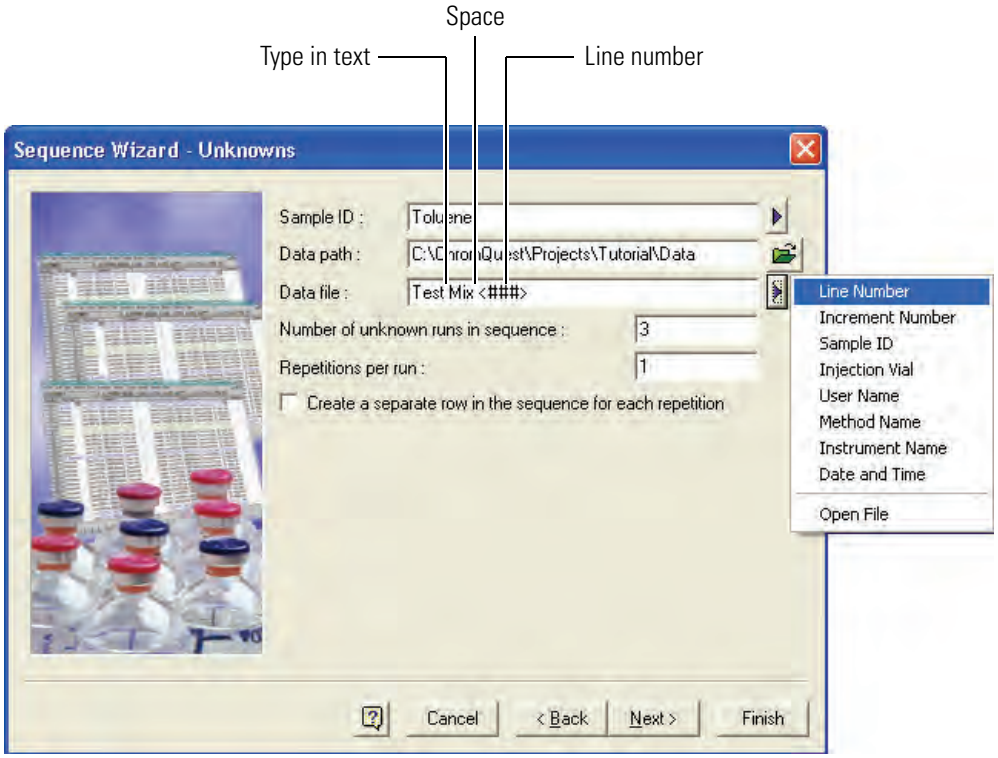



3. In the Unknowns page, keep the settings at their defaults except for the following changes, as shown in Figure 161:
   a. Type **Toluene** in the Sample ID box.
   b. Verify that the appropriate Data path is displayed.

      **Note** If you are using the Tutorial project to organize your data, verify that the following data path is displayed: D*rive:\ChromQuest\Projects\Tutorial\Data.*

   c. Give unique names to the data files in this sequence table:
      i. Type **Test Mix** in the Data file box. Then add a space.
      ii. Click the blue arrow next to the Data file box.
      iii. From the pop-up menu, choose **Line Number**.
   d. Type **3** in the Number of unknown runs in sequence box.
   e. Click **Next** to open the Autosampler page of the Sequence Wizard.

**Figure 161.** Unknowns page




4. In the Autosampler page, make the entries described below and shown in Figure 162:

   a. In the Unknown vials of sequence area, type **A;1** (A semicolon 1)in the First Vial box.

   **Note** You must type a semicolon between the tray type and the tray location.

   b. In the Calibration vials of sequence area, type **A;1** in the First Vial box.

   All the injections for this sequence will be made from the standard 1.8 mL vial in the A1 position of the standard tray.

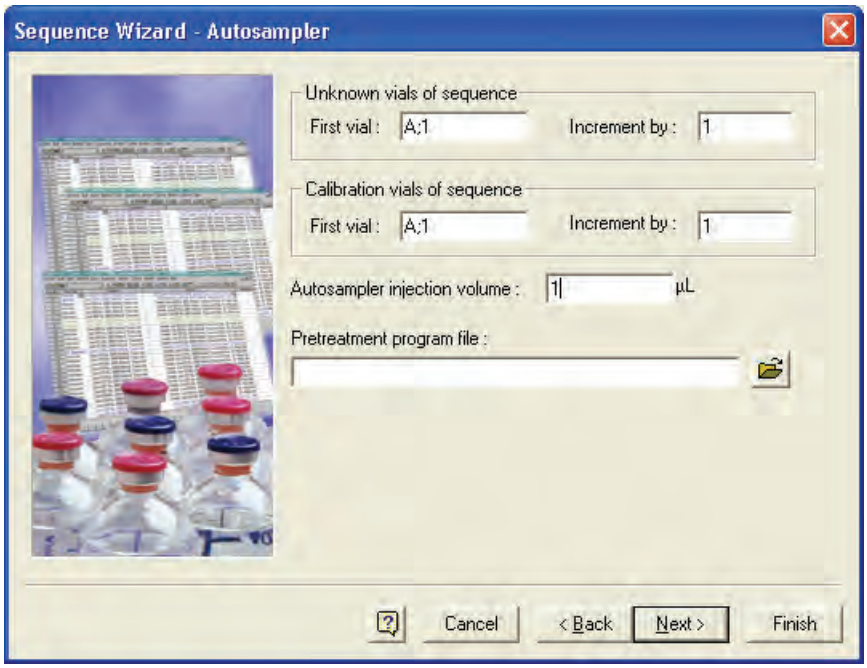
   c. In the Autosampler injection volume box, enter an injection volume in µL:

   - Type **1** if you are using a 5 cm LightPipe flow cell.
   - Type **5** if you are using a standard 1 cm flow cell.

   d. Verify that the Pretreatment program file box is empty.

   e. Click **Next** to open the Calibration page of the Sequence Wizard.

**Figure 162.** Autosampler page




5. In the Calibration page, keep the settings at their defaults except for the following entries and selections, as shown in Figure 163:

   a. In the Number of calibration levels box, type **3**.

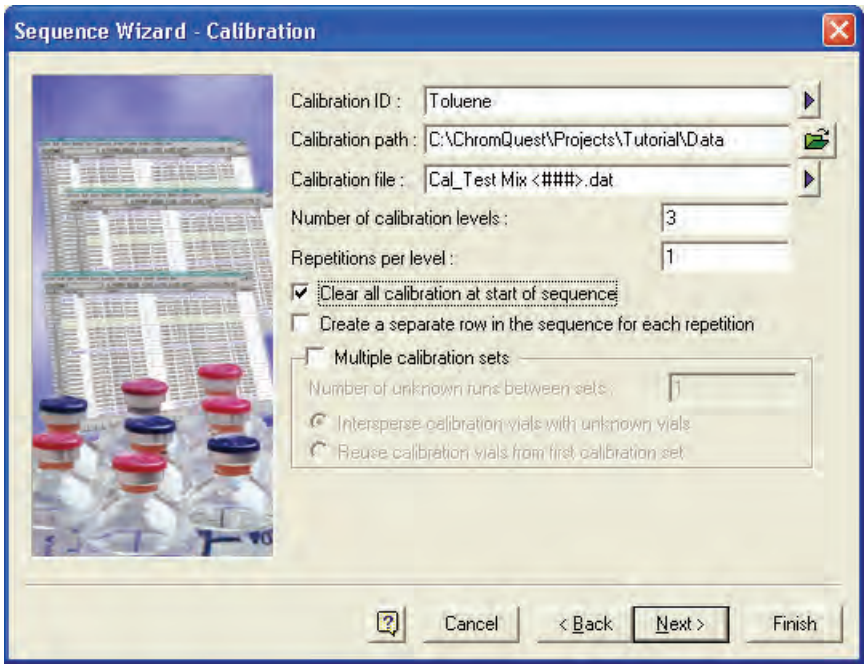
      The peak table that you created while performing the tutorial contained in Chapter 8 has three calibration levels. To simulate three concentration levels, you will inject variable amounts of sample as described in the topic "Changing the Injection Volume" on page 191.

   b. Select the **Clear all calibration at start of sequence** check box.

      This allows ChromQuest to clear the absorbance values in the calibration table of the method at the beginning of a sequence run or a sequence reprocessing.

   c. Click **Next** to open the Reports page of the Sequence Wizard.

**Figure 163.** Calibration page




6. In the Reports page, keep the settings at their defaults except for the following changes in the Summary area, as shown in Figure 164:

   - Select the **Include unknown runs in summary report** check box.
   - Select the **Include calibration runs in summary report** check box.

7. Click **Finish**.

   The unedited sequence table appears. See Figure 165.

**Figure 164.** Reports page

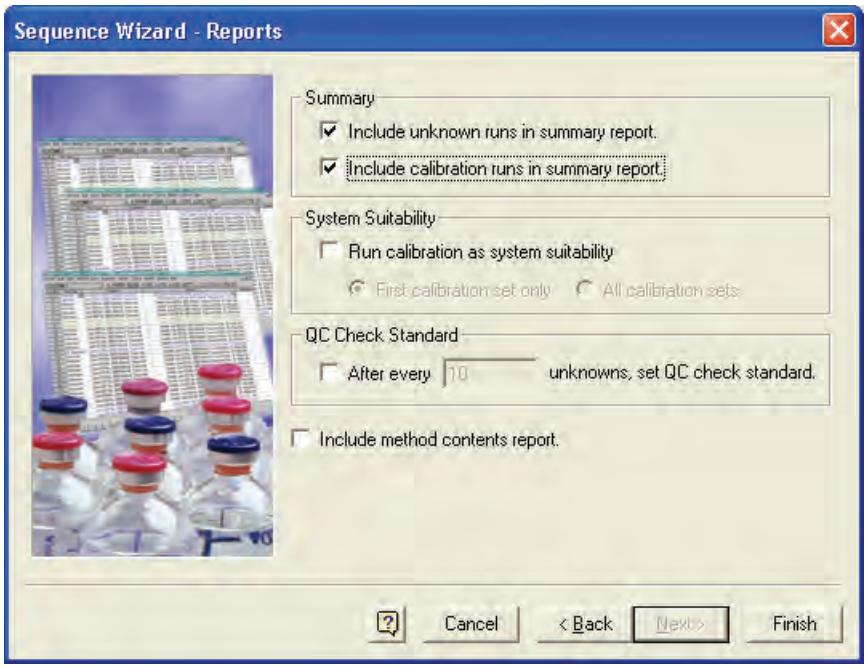
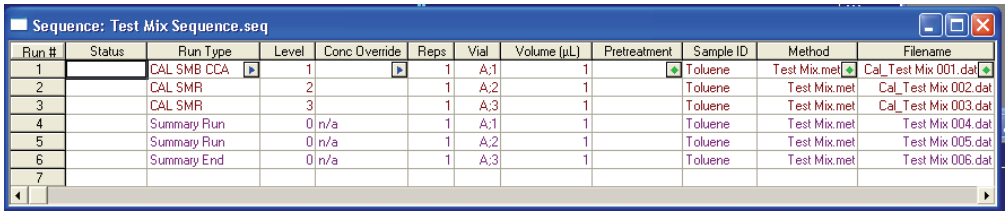



**Figure 165.** Unedited sequence table




8. Save the sequence table with the name, Test Mix Sequence.seq:

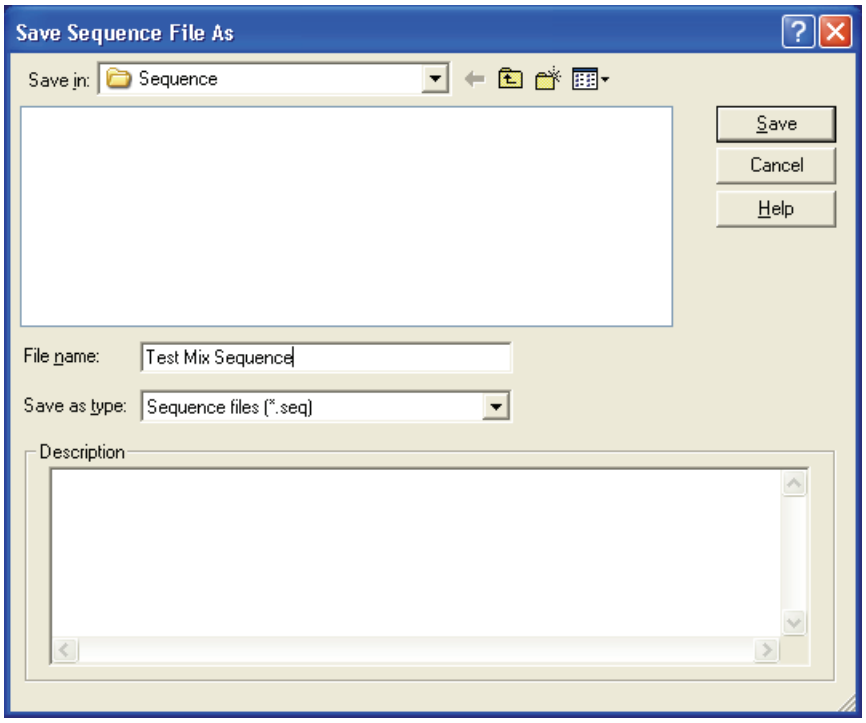
   a. Choose **File > Sequence > Save As** to display the Save Sequence File As dialog box, shown in Figure 166.

   b. Type **Test Mix Sequence** in the File Name box.

   c. Click **Save**.

   ChromQuest appends the file extension .seq to the sequence name.

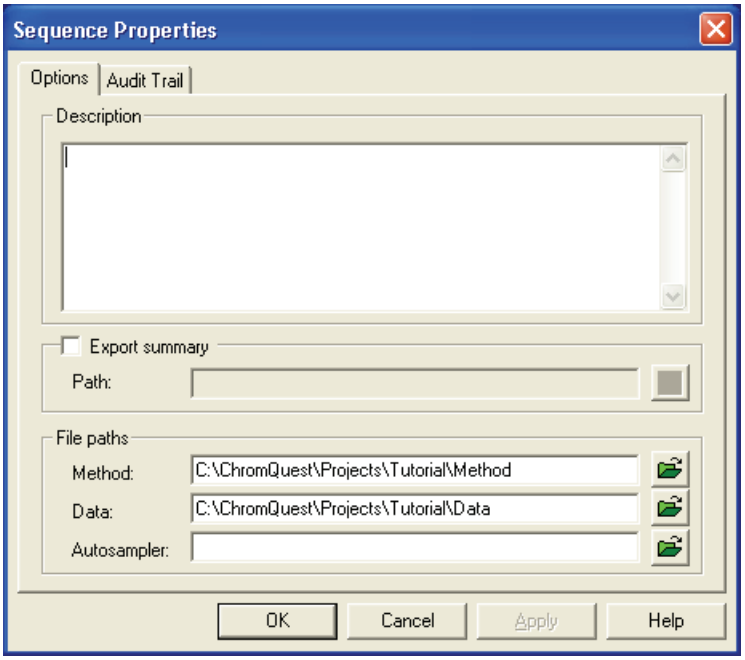
**Figure 166.** Save Sequence File As dialog box




9. Verify the file folders for the sequence:
   a. From the Instrument window, choose **Sequence > Properties**.

      The Sequence Properties dialog box appears. See Figure 167.

      **Figure 167.** Sequence Properties dialog box



b. Verify that the method and the data files are in the appropriate project folder. Then, close the Sequence Properties dialog box.

Keep the sequence table open on the view screen and proceed to the next topic, "Modifying the Sequence Table" on page 188.

# Modifying the Sequence Table

You can create a basic sequence table using the Sequence Wizard. If you want to turn off the solvent flow from the pump or turn off the lamps at the end of a sequence run, you must modify the sequence table.

If your sequence is not already open, click the **Edit Sequence**  button to open it.

This section contains the following procedures for common modifications made to sequence tables:

- Deleting Unused Columns from the Sequence Table
- Changing the Vial Locations
- Changing the Injection Volume
- Adding a Shutdown Line
- Adding an Action

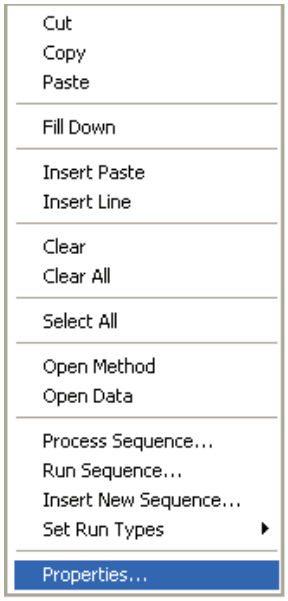
# Deleting Unused Columns from the Sequence Table

By default, the Sequence table contains 22 columns. To make it easier to work with the table, delete some of the columns from view.

### ❖ To delete unused columns from the sequence table

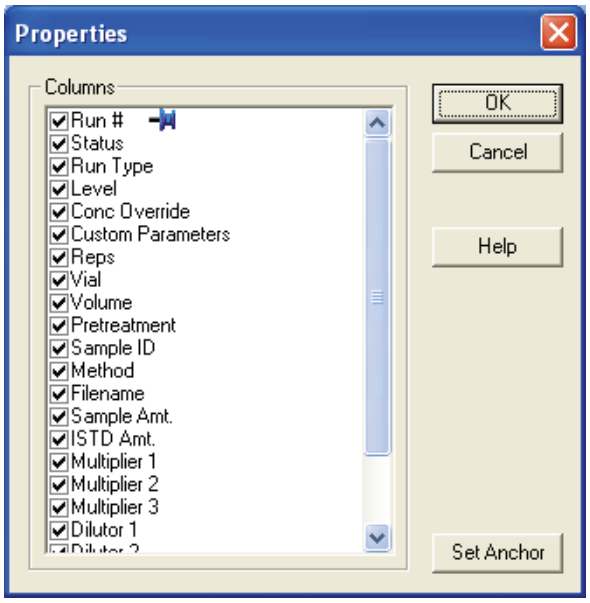
1. Right-click in the sequence table to open its shortcut menu. See Figure 168.

   **Figure 168.** Sequence shortcut menu




2. From the shortcut menu, choose **Properties** to open the Properties dialog box. See Figure 169.

   **Figure 169.** Properties dialog box



3. Clear the check boxes for all the columns except for those listed below.

   - Run #
   - Status
   - Run Type
   - Level
   - Reps
   - Vial
   - Volume
   - Sample ID
   - Method
   - Filename
   - Action

4. Click **OK** to exit the Properties dialog box.

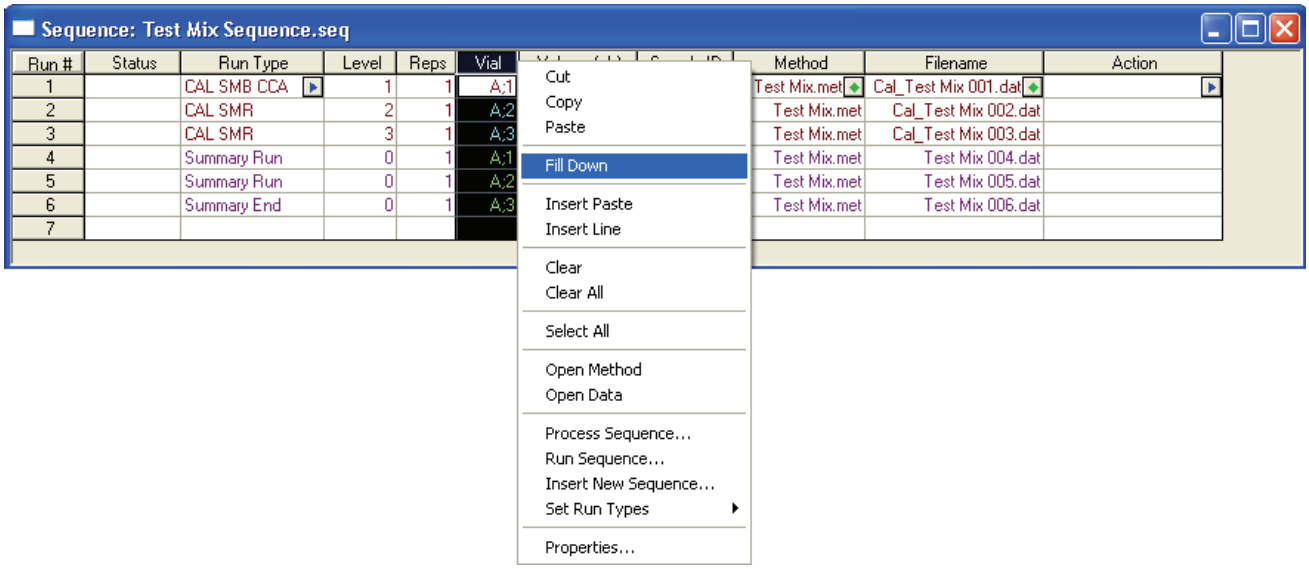
## Changing the Vial Locations

In the next chapter, you make a sequence of injections from the same vial. Therefore, you need to modify the vial locations listed in the current sequence table, Test Mix Sequence.seq.

❖ **To change the vial locations and make the injections from the same vial**

1. Select the Vial column by clicking its header.
2. Right-click in the sequence table to open the shortcut menu, as shown in Figure 170.

**Figure 170.** Sequence shortcut menu




3. From the shortcut menu (see Figure 170), choose **Fill Down** to open the Fill Down dialog box.

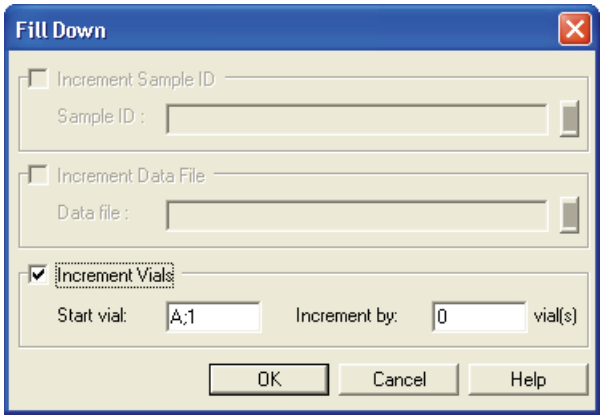
4. Except for the following change in the Increment Vials area, leave the settings in the Fill Down dialog box at their defaults, as shown in Figure 171:

   a. In the Increment by box, highlight the number 1, and then type **0**.

   b. Click **OK**.

   A;1 is now the only vial listed in the Vial column.

**Figure 171.** Fill Down dialog box




5. To save the sequence, click the **Save** button in the Command toolbar, and then choose **Save Sequence**.

## Changing the Injection Volume

In this tutorial, you use one vial for all your injections. Instead of injecting a set of calibration standards to create your calibration curve, you inject a variable amount of sample solution.

### ❖ To change the injection volumes in your sequence table

1. Click the first cell in the Volume column. Then enter an appropriate injection volume for the first calibration level:
   - If you are using a 5 cm LightPipe flow cell, type **0.5.**
   - If you are using a standard 1 cm flow cell, type **2.5**.
2. Click the second cell in the Volume column. Then, enter an appropriate injection volume for the second calibration level:
   - If you are using a 5 cm LightPipe flow cell, type **1**.
   - If you are using a standard 1 cm flow cell, type **5.0**.
3. Click the third cell in the Volume column. Then, enter an appropriate injection volume for the third calibration level:
   - If you are using a 5 cm LightPipe flow cell, type **1.5**.
   - If you are using a standard 1 cm flow cell, type **7.5**.

As the calibration data files in the sequence are acquired, the injection volumes that you entered will create a calibration curve proportional to the values entered in the peak table. See page 157, which describes the concentration entries in the peak table.

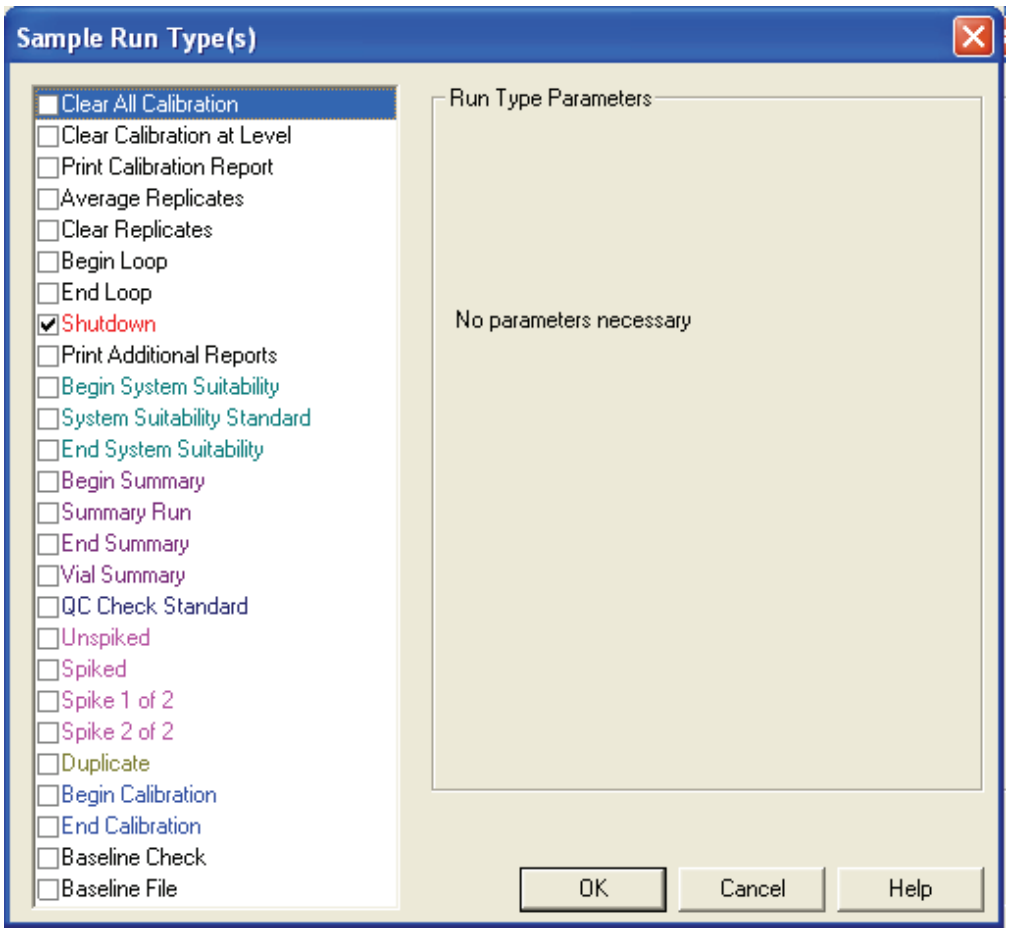
## Adding a Shutdown Line

At the end of a sequence run, you might want to turn off the mobile phase flow to conserve solvent, the lamps to extend their lifespan, or both. To turn off the mobile phase flow or the lamps, you must create a shutdown method and add a shutdown line to the end of your sequence table. You created a shutdown method named Shutdown.met while performing the tutorial "Creating a Shutdown Method" on page 100.

### ❖ To add a shutdown line at the end of your sequence table

1. In the seventh row of the sequence table (see Figure 170), click the blue arrow in the Run Type column.
2. In the Sample Run Type(s) dialog box (see Figure 172), select the **Shutdown** check box.
3. Click **OK** to exit the Sample Run Types dialog box.

**Figure 172.** Sample Run Type dialog box



4. In the Method column of the sequence table (see Figure 170), click the green arrow.

   The Open Method File dialog box appears.

5. Select the Shutdown.met shutdown method, and then click **Open**.

   Row seven of your sequence table now contains a Shutdown run type and lists the Shutdown.met. The entire row is highlighted in red.

6. Save the sequence table by choosing **File > Sequence > Save**.

## Adding an Action

The Action feature automates a variety of operations, such as setting off an alarm is a baseline check fails or performing a shutdown if a hardware status error occurs.

### ❖ To shut down the LC in the event of a hardware error

1. In row 1 of the sequence table (see Figure 170), click the blue arrow in the Action column.

   The Action dialog box, shown in Figure 173, appears.

2. Make the following selections in the Action dialog box:

   a. In the Test column, select **Hardware Status**.

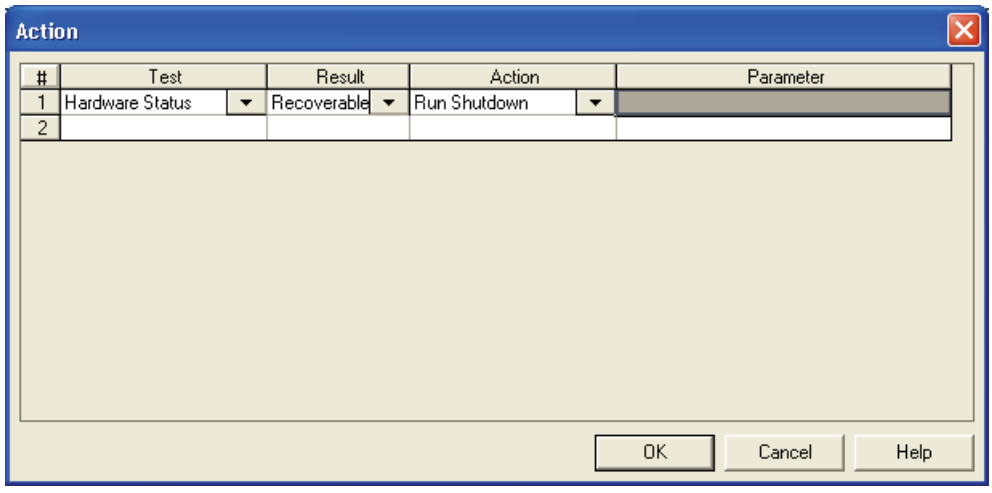
   b. In the Result column, select **Fail**.

   c. In the Action column, select **Run Shutdown**.

3. Click **OK** to close the dialog box.

4. Save the sequence by choosing **File > Sequence > Save**.

**Figure 173.** Action dialog box, showing a Hardware Status test



# Creating a New Sequence Summary Template

By default, the basic sequence created with the Sequence Wizard uses the sequence summary template Summary.tpl, which reports the concentrations of the named peaks for the first wavelength listed in the Channel Selection list. ChromQuest contains several other sequence summary templates from which to choose, but none of these templates reports the concentrations of your analytes for two or more wavelengths. Therefore, to print a summary of the results for both of your wavelength channels, you must create your own sequence summary template and then add it to your sequence table.

To create your own customized sequence summary report and add it to your sequence table, perform the following procedures:

1. Opening the Sequence Editor
2. Using the Table Wizard
3. Selecting a Sequence Summary Template

## Opening the Sequence Editor

Create your own sequence summary template by using the Sequence Editor. to modify one of the standard sequence summary templates. The sequence summary template *Summary.tpl* reports the concentrations of named peaks at one wavelength. This summary report can be easily modified to report the concentrations of your named peak at two wavelengths.

### ❖ To open the sequence summary template for editing

1. From the Instrument window, choose **File > Advanced Reports > Open**.

   The **Open Advanced Report File** dialog box, shown in Figure 174, appears.

2. Select the sequence summary report template Summary.tpl.
3. Click **Open**.

   The Summary template opens in the Template Editor window, as shown in Figure 175.

> **Tip** Double-click the formula cells (EX.R...) to view the formulas.

**Figure 174.** Open Advance Report File dialog box

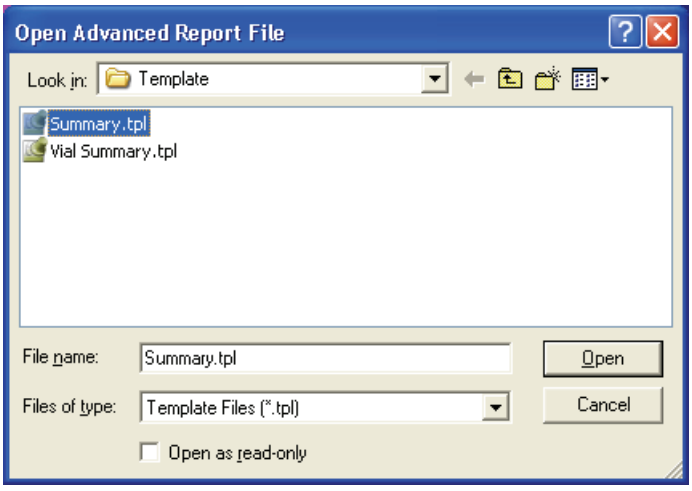
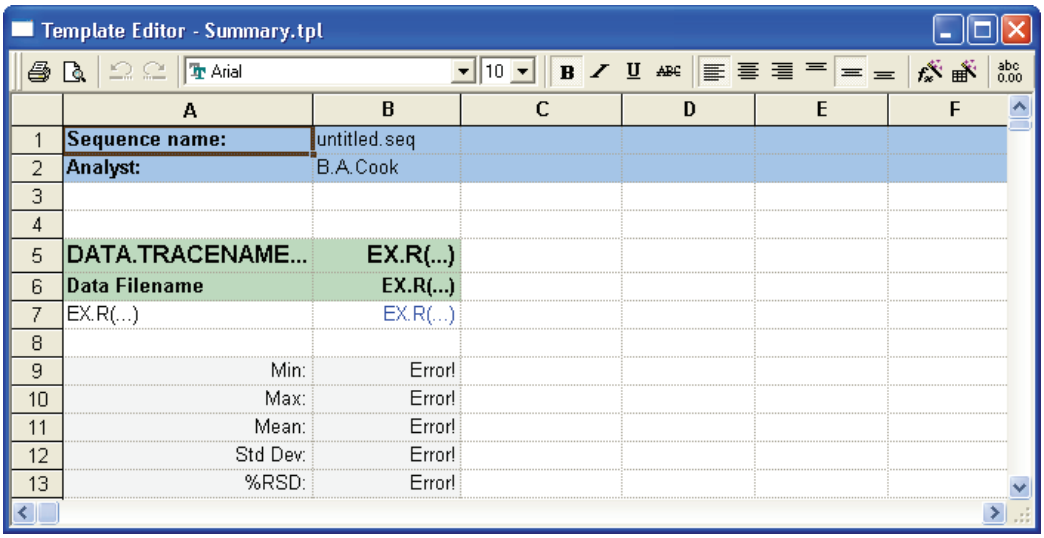



**Figure 175.** Template Editor, showing the sequence summary template, Summary.tpl



## Using the Table Wizard

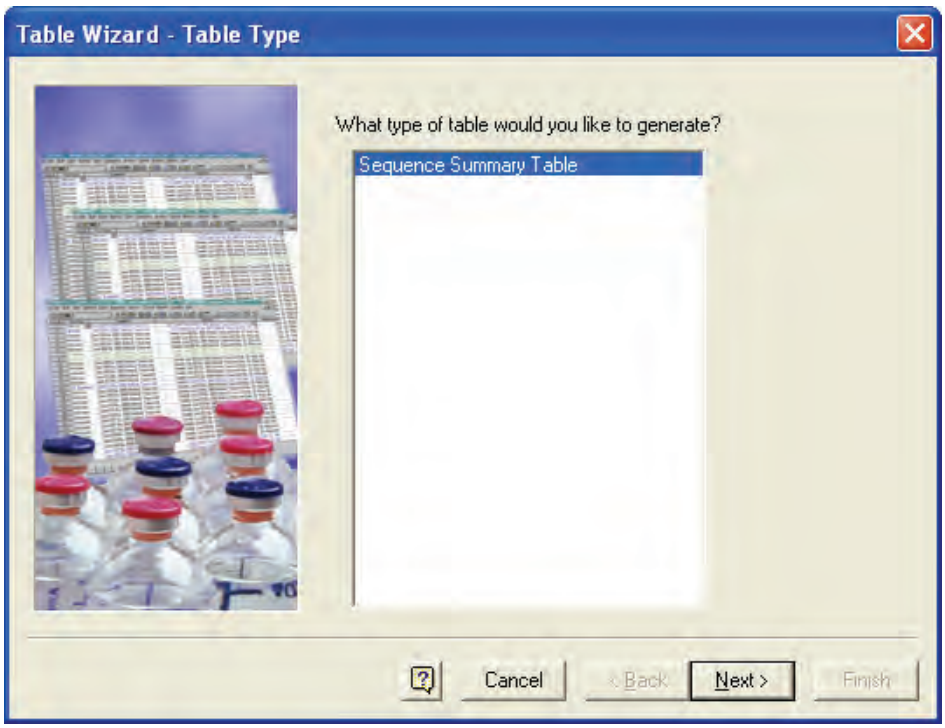
Now that you have the sequence summary template (Summary.tpl) open for editing, add an additional table to report the results of the 260 nm analysis channel.

### ❖ To add an additional table to the sequence summary template

1. Ensure that your method Test Mix.met is the active method, so that the traces are available.
2. Place your cursor in cell **A15** of the sequence summary template (Summary.tpl).
3. Click the **Table Wizard** button.

   The Table Wizard appears. See Figure 176.

   **Figure 176.** Table Wizard – Table Type page

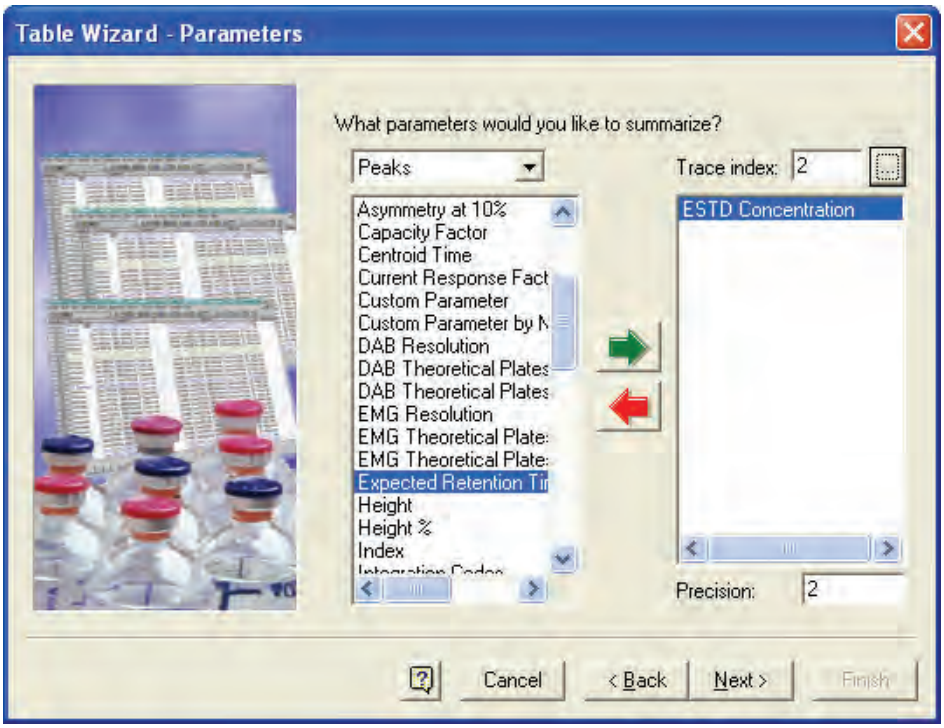



4. Click **Next.**

   The Parameters page appears.

5. In the Parameters page, make the following selections, as shown in Figure 177:
   a. Click the **Trace Index** button to display the available traces.
   b. Select the 260 nm wavelength (trace index 2).
   c. Click **Select Trace**.
   d. Double-click **ESTD Concentration** to add it to the list of parameters on the right.

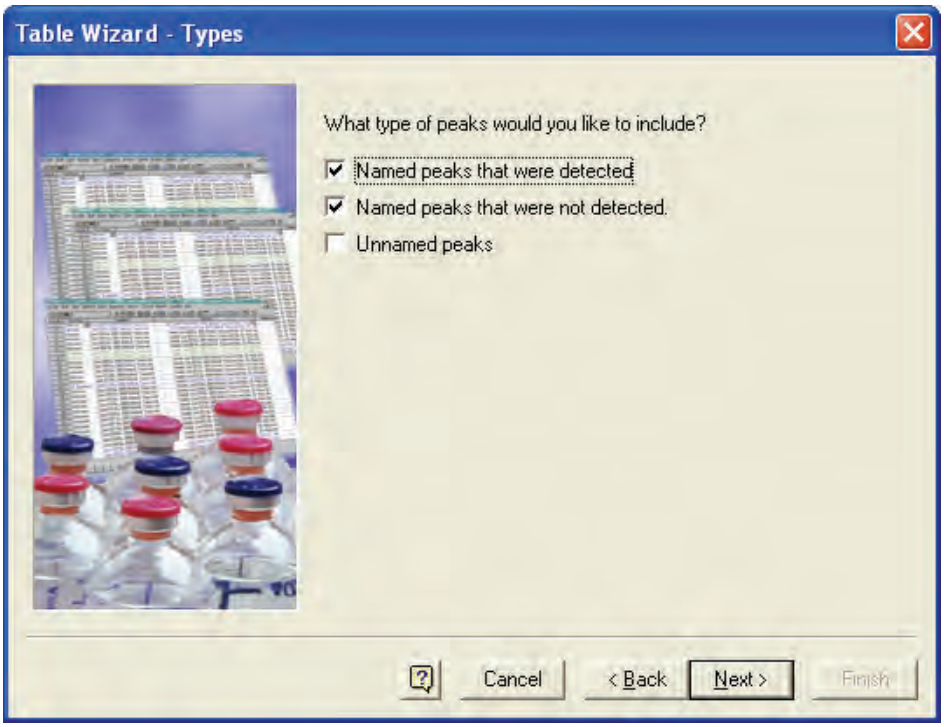
**Figure 177.** Table Wizard – Parameters page




e. Click **Next**.

The Types page appears. See Figure 178.

**Figure 178.** Table Wizard – Types page with the default settings

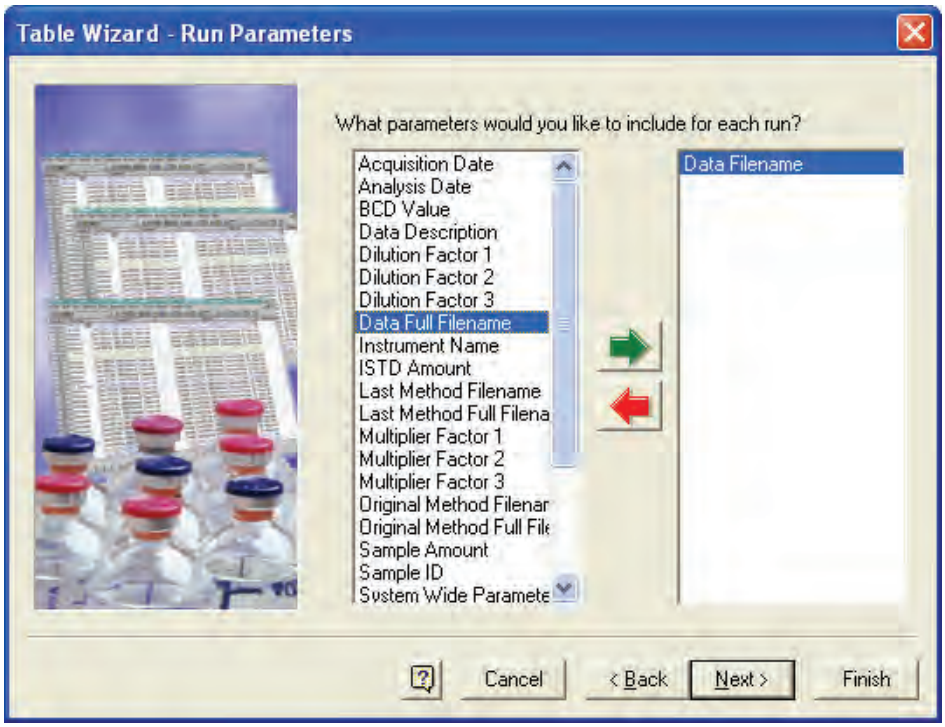


6. Keep the parameters in the Types page at their default settings, as shown in Figure 178, and then click **Next**.

   The Run Parameters page appears.

7. In the Run Parameters page, double-click **Data Filename** to add it to the box on the right, as shown in Figure 179, and then click **Next**.

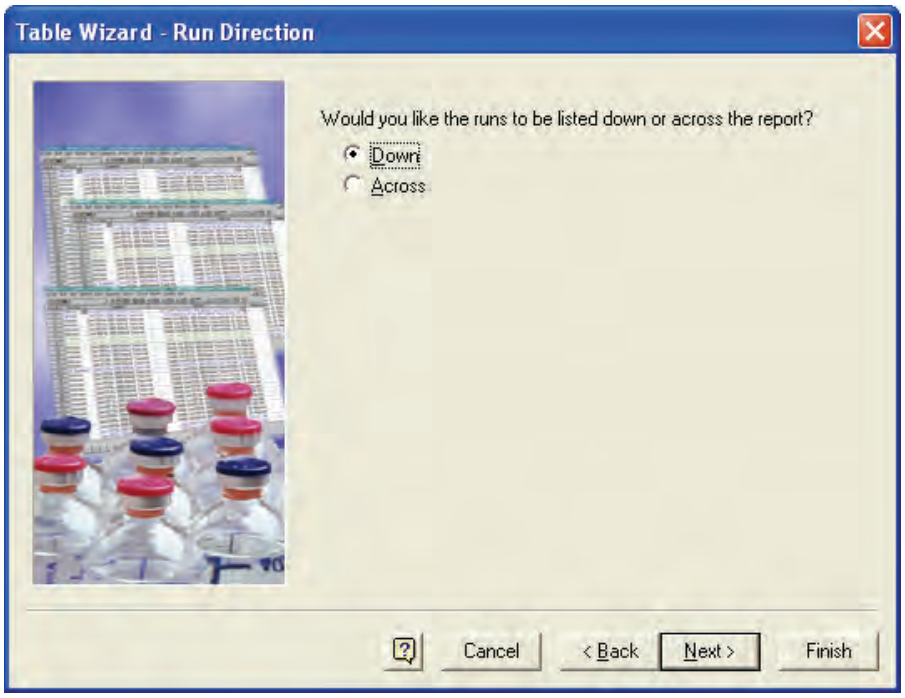
   **Figure 179.** Table Wizard – Run Parameters page with Data Filename selected




   The Run Direction page, shown in Figure 180, appears.

8. In the Run Direction page, leave the Run Direction at its default setting of Down, and then click **Next**.

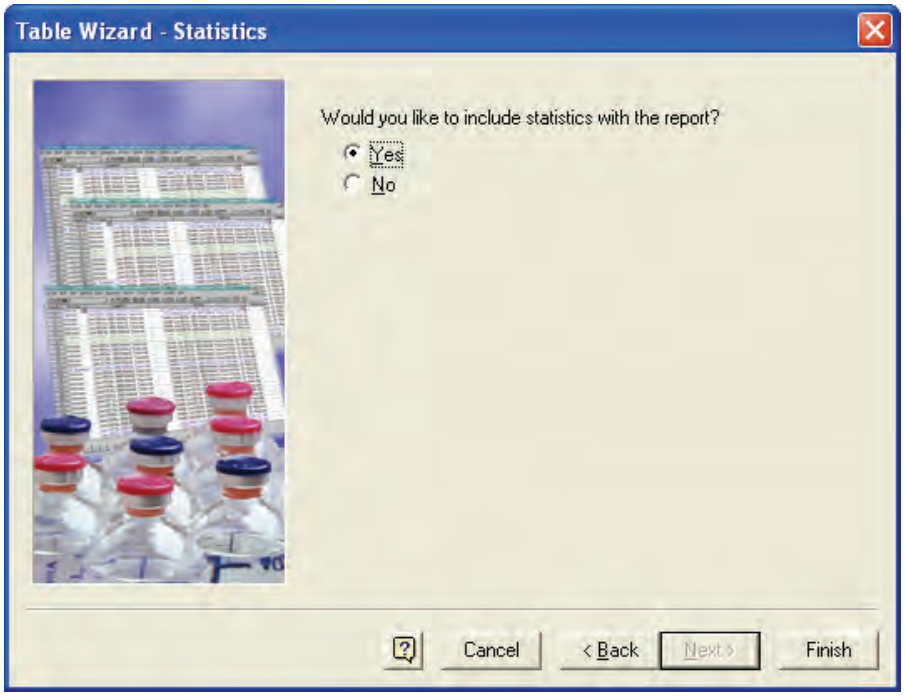
**Figure 180.** Table Wizard – Run Direction page with the default setting of Down




The Statistics page, shown in Figure 181, appears.

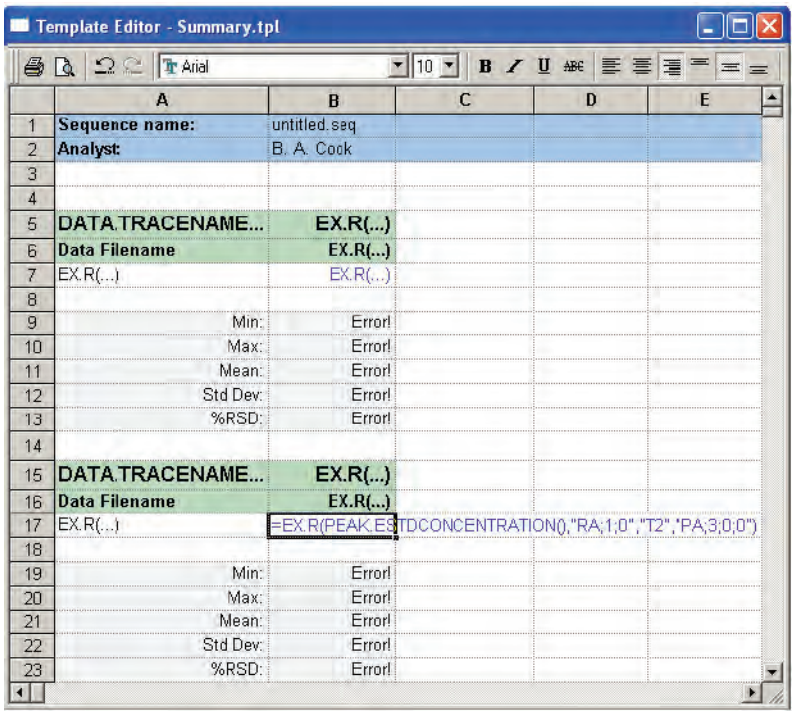
9. In the Statistics page, leave the selection at its default setting of **Yes**, as shown in Figure 181, and then click **Finish**.

**Figure 181.** Table Wizard – Statistics page with the default setting of Yes



Your new template appears. See Figure 182.

**Figure 182.** New template with the external standard concentration formula for second trace



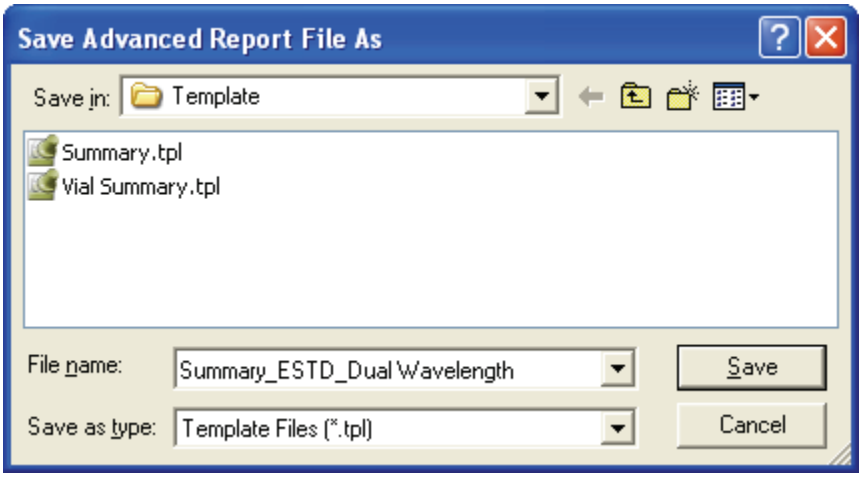

10. Save the new template with the name Summary_ESTD_Dual Wavelength.tpl:
    a. Choose **File > Advanced Reports > Save As.**

       The Save dialog box, shown in Figure 183, appears.

    b. In the File Name box, type **Summary_ESTD_Dual Wavelength**.

    c. Click **Save.**

       **Figure 183.** Save As dialog box

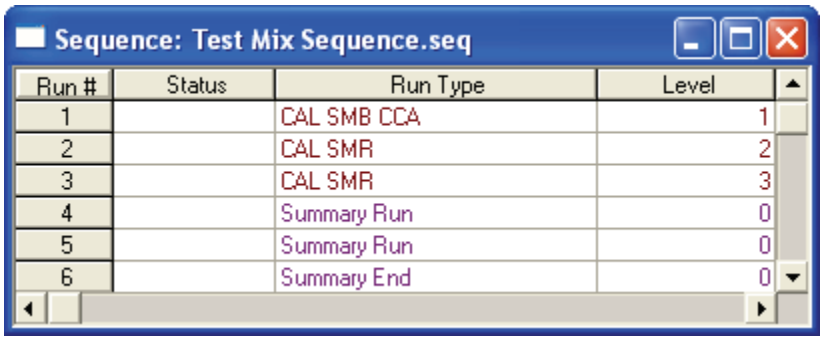


# Selecting a Sequence Summary Template

❖ **To select the sequence summary template**

1. Double-click in the Run Type cell that contains the Begin Summary (SMB) run type in row #1. See Figure 184.

   **Figure 184.** Test Mix Sequence table, showing Run Type column

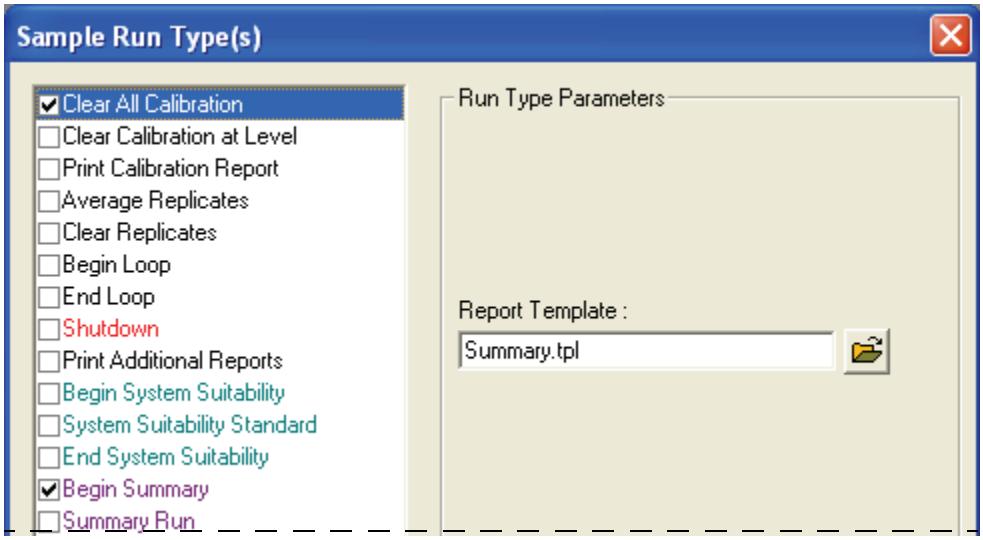



   The Sample Run Types dialog box appears. See Figure 172.

2. In the Sample Run Types dialog box, select the **Begin Summary** check box.

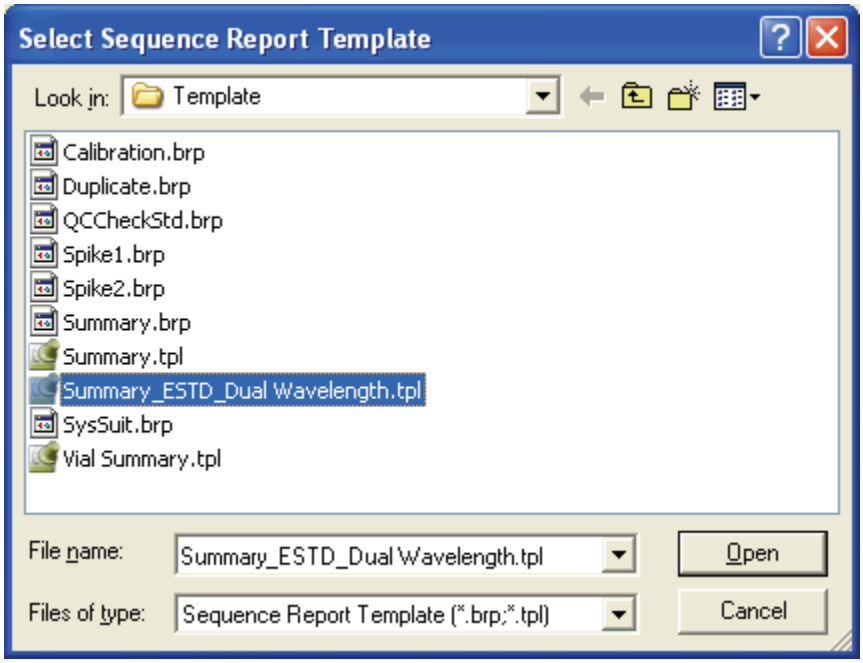
   The Report Template box shows the current report template. See Figure 185.

   **Figure 185.** View of the current report template selection




3. Click the file folder next to the Report Template box to open the Select Sequence Report Template dialog box. See Figure 186.

**Figure 186.** Select Sequence Report Template dialog box




4. Select the **Summary_ESTD_Dual Wavelength.tpl** template that you created from the list of templates.
5. Save the sequence table by choosing **File > Sequence > Save**.

# Running and Reprocessing a Sequence

In this tutorial, you learn how to start a sequence run. In addition, you learn how to reprocess the data files acquired by running the sequence and review the calibration table contained in your method.

To start a sequence run, you must be working in the Online Instrument window. To reprocess a sequence file, you can be working in either the Online or Offline Instrument window.

**Contents**

# Starting a Sequence Run

To start a sequence run, you must be working in the Online Instrument window. If you have not already created a sequence table listing your sample set, see Chapter 10, "Creating a Sequence Table," for information on creating a sequence table.

### ❖ To start a sequence run

1. Ensure that your standard tray contains a vial in the A1 vial location.
2. From the online Instrument window toolbar, click the **Sequence Run** button.

   The Sequence Run dialog box appears.
3. Set the parameters to the settings that are shown in the following table and in Figure 187.

| Parameter | Setting | Result |
|---|---|---|
| Sequence Information | | |
| • Sequence Name | Drive:\ChromQuest\Projects\Tutorial\Sequence\Test Mix Sequence.seq<br><br>Your directory might be different. Browse to the appropriate directory. | Lists the sequence that will be run |
| Printing | | |
| • Printing | ☑ Print Method Reports | Specifies that the method custom reports will be printed after the data file is acquired |
| | ☑ Print Sequence Reports | Specifies that your sequence summary report will be printed at the end of the sequence run |

4. Click **Start**.

   A warning message appears because your sequence table contains the same vial locations for your calibration standards as it does for your unknowns. See Figure 188.
5. In the Overwriting Sample Type dialog box, click **OK To All**.

   The sequence run commences. As each data file is acquired, the method custom report that you created while performing the tutorial contained in Chapter 9, "Adding a Custom Report to the Method," is printed.

**Figure 187.** Run Sequence dialog box

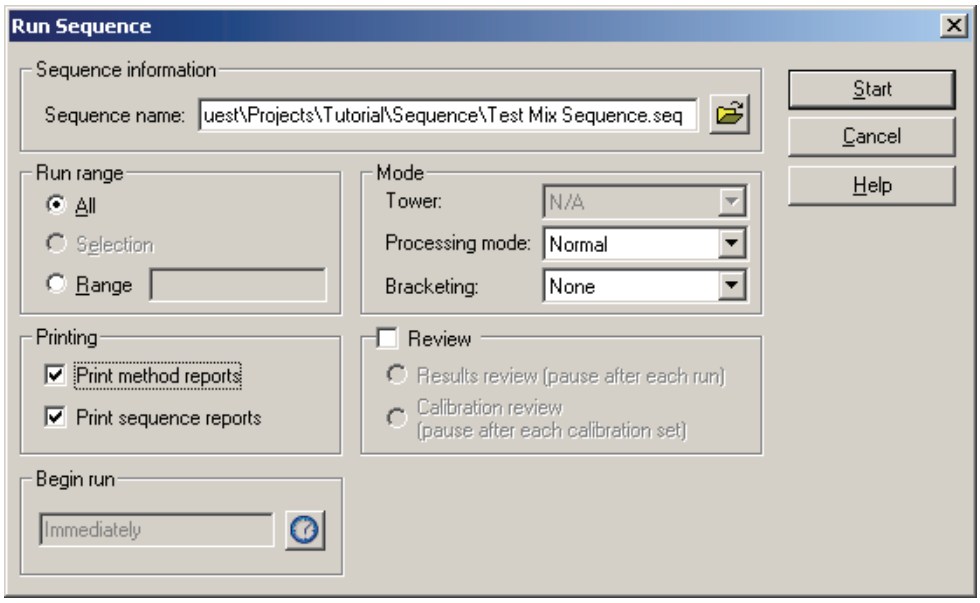
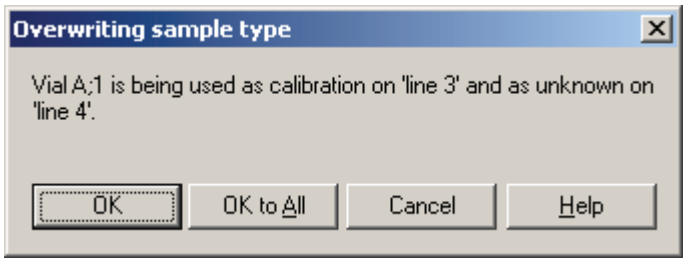


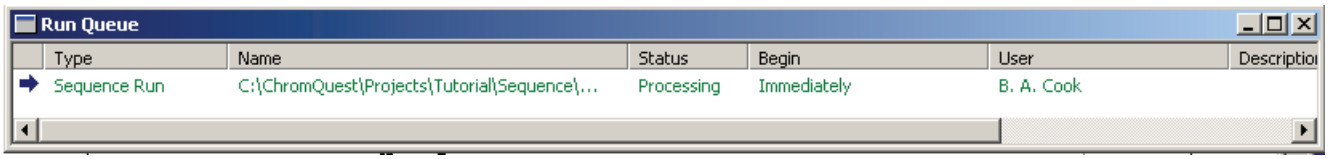

**Figure 188.** Overwriting Sample Type warning box




6. To see which sequences or single runs are currently being processed, click the **Run Queue** button.

   The Run Queue window appears. See Figure 189.

**Figure 189.** Run Queue window, showing the sequence in progress




After ChromQuest acquires the data file Test Mix 003.dat, it prints the sequence summary report that you created while performing the tutorial contained in Chapter 10, "Creating a Sequence Table," See Figure 190.

**Figure 190.** Sequence summary report containing data for two wavelengths

## Thermo Fisher Scientific Laboratories

**ChromQuest 5.0**
**Sequence Summary Report**

| | |
|---|---|
| **Sequence name:** | **Test Mix Sequence** |
| **Analyst** | **B. A. Cook** |

| **PDA-230 nm** | | **Toluene_230nm** |
|---|---|---|
| **Data Filename** | | **ESTD** |
| Cal_Test Mix 001.dat | | 1.00 |
| Cal_Test Mix 002.dat | | 2.00 |
| Cal_Test Mix 003.dat | | 3.00 |
| Test Mix 001.dat | | 2.03 |
| Test Mix 002.dat | | 2.04 |
| Test Mix 003.dat | | 1.99 |
| | Min: | 1.00 |
| | Max: | 3.00 |
| | Mean: | 2.01 |
| | Std Dev: | 0.58 |
| | %RSD: | 28.75 |

| **PDA-260 nm** | | **Toluene_260nm** |
|---|---|---|
| **Data Filename** | | **ESTD** |
| Cal_Test Mix 001.dat | | 1.00 |
| Cal_Test Mix 002.dat | | 2.00 |
| Cal_Test Mix 003.dat | | 3.00 |
| Test Mix 001.dat | | 2.04 |
| Test Mix 002.dat | | 2.04 |
| Test Mix 003.dat | | 2.00 |
| | Min: | 1.00 |
| | Max: | 3.00 |
| | Mean: | 2.01 |
| | Std Dev: | 0.63 |
| | %RSD: | 31.43 |

7. After the sequence run has ended (approximately 30 min), review the information in the title bar.

   The current method is the shutdown method named Shutdown.met.

8. Open the Instrument Status window by choosing **Control > Instrument Status**, and verify that the lamps are off and that the flow rate is set to zero.

9. Open the method Test Mix.met by choosing **File > Method > Open**. Select Test Mix.met from the list, and then click **Open**.

# Reviewing the Peak Calibration

After you have completed running all of the calibration standards for your method, you can review the calibration curve and associated data.

### ❖ To review the peak calibration information

1. Click the **Review Peak Calibration** button to review the calibration information contained in the method Test Mix.met for the two wavelengths: 230 nm and 260 nm.
2. Click the Toluene_230nm peak in the Peak list to view its calibration information. See Figure 191.
3. Click the Toluene_260nm peak in the Peak list to view its calibration information. See Figure 192.

   The calibration curve for the selected peak appears in the lower left corner of the window. The box in the lower right corner of the window displays information about the calibration data.

**Figure 191.** Review Peak Calibration window, showing the calibration data for the peak named Toluene_230 nm

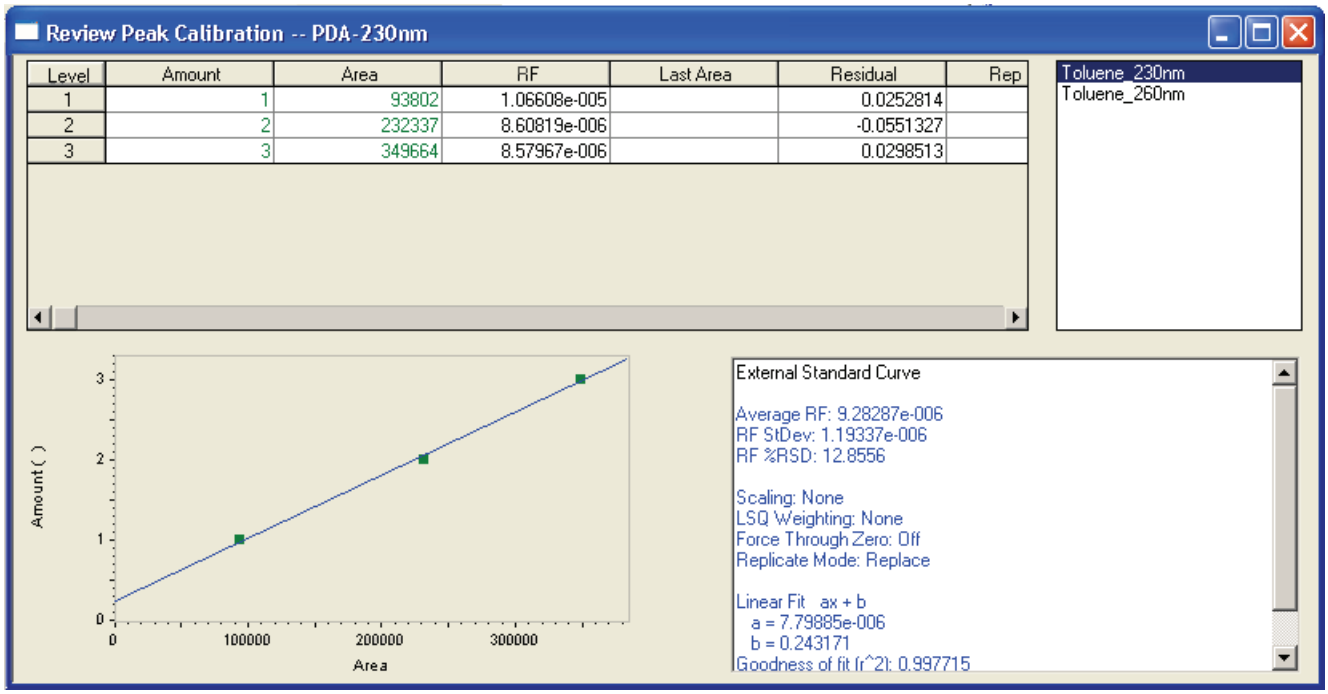
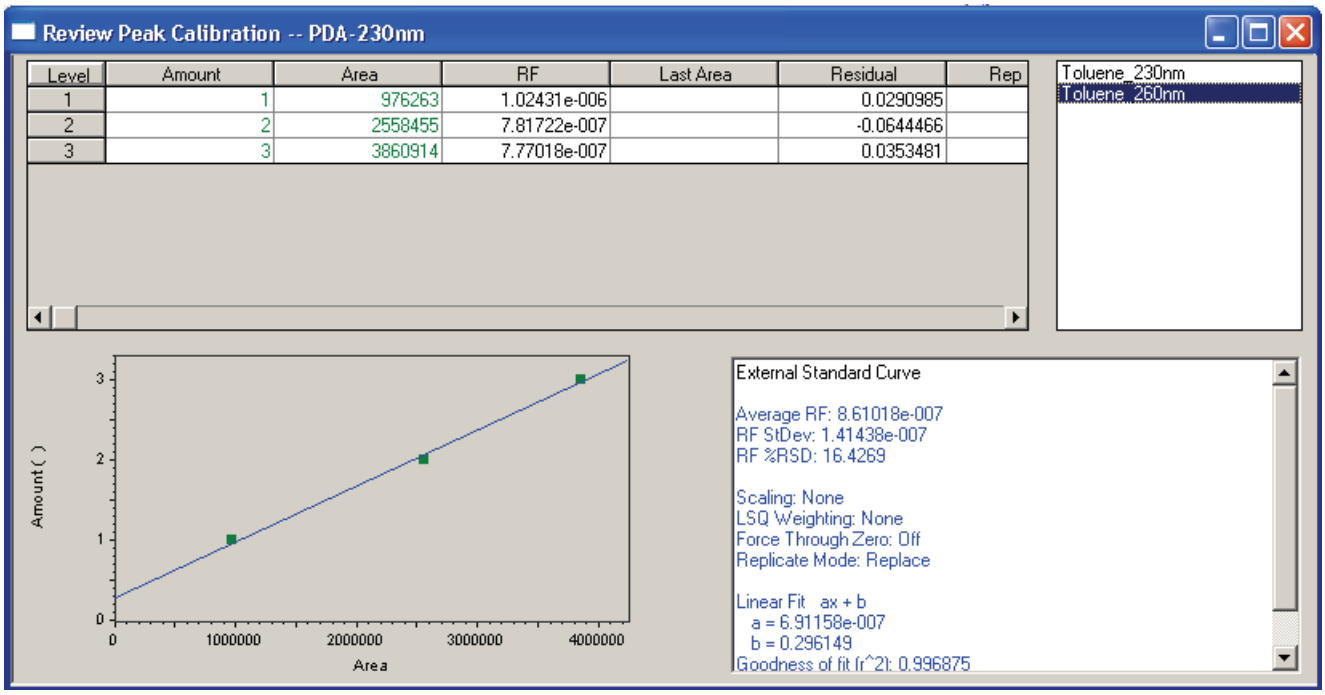


**Figure 192.** Review Peak Calibration window, showing the calibration data for the peak named Toluene_260 nm




# Reprocessing a Sequence Run

❖ **To review the results of your sequence run**

1. Choose **Sequence > Process** or click the **Sequence Process** button.

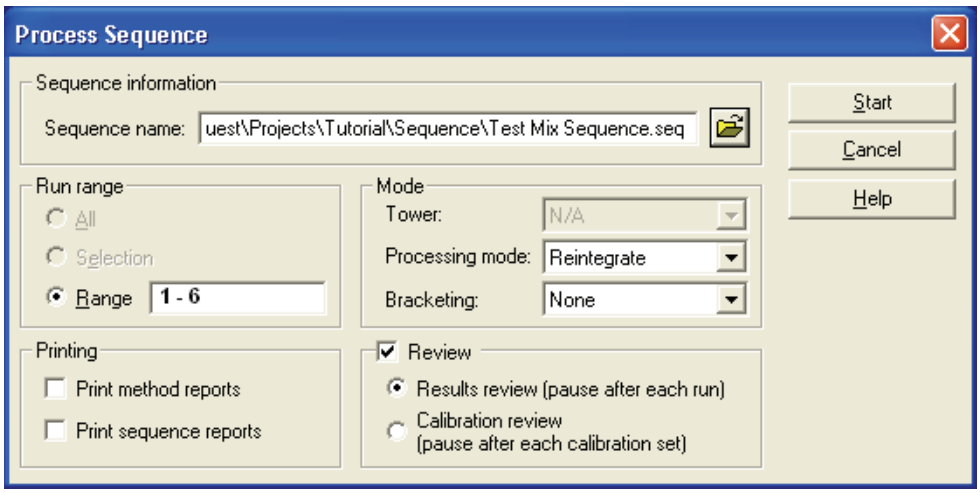
   The Process Sequence dialog box appears.

2. Set the parameters in the dialog box to the settings that are shown in the following table and in Figure 193.

   Instead of selecting a run range of **All** as you did when you originally ran your sequence, select a range consisting of rows 1 through 6. You exclude row 7 from the selection because it contains a shutdown run without a specified file name.

| Parameter | Setting | Result |
|---|---|---|
| Sequence Information | | |
| • Sequence Name | Drive:\ChromQuest\Projects\Tutorial\Sequence\Test Mix Sequence.seq | Lists the sequence that will be processed |
| Run Range | | |
| • Run Range | ◉ Range | |
| | 1-6 | Specifies the reprocessing of rows 1 through 6 |
| Mode | | |
| • Processing Mode | Reintegrate | Specifies that the data files will be reintegrated |
| Review | | |
| • Review | ☑ Review | Turns on Review |
| | ◉ Results Review (Pause After Each Run) | Pauses after each run. To review the next data file, you must click the green down arrow at the bottom of the Instrument window |

**Figure 193.** Process Sequence dialog box, showing entries for reviewing your sequence



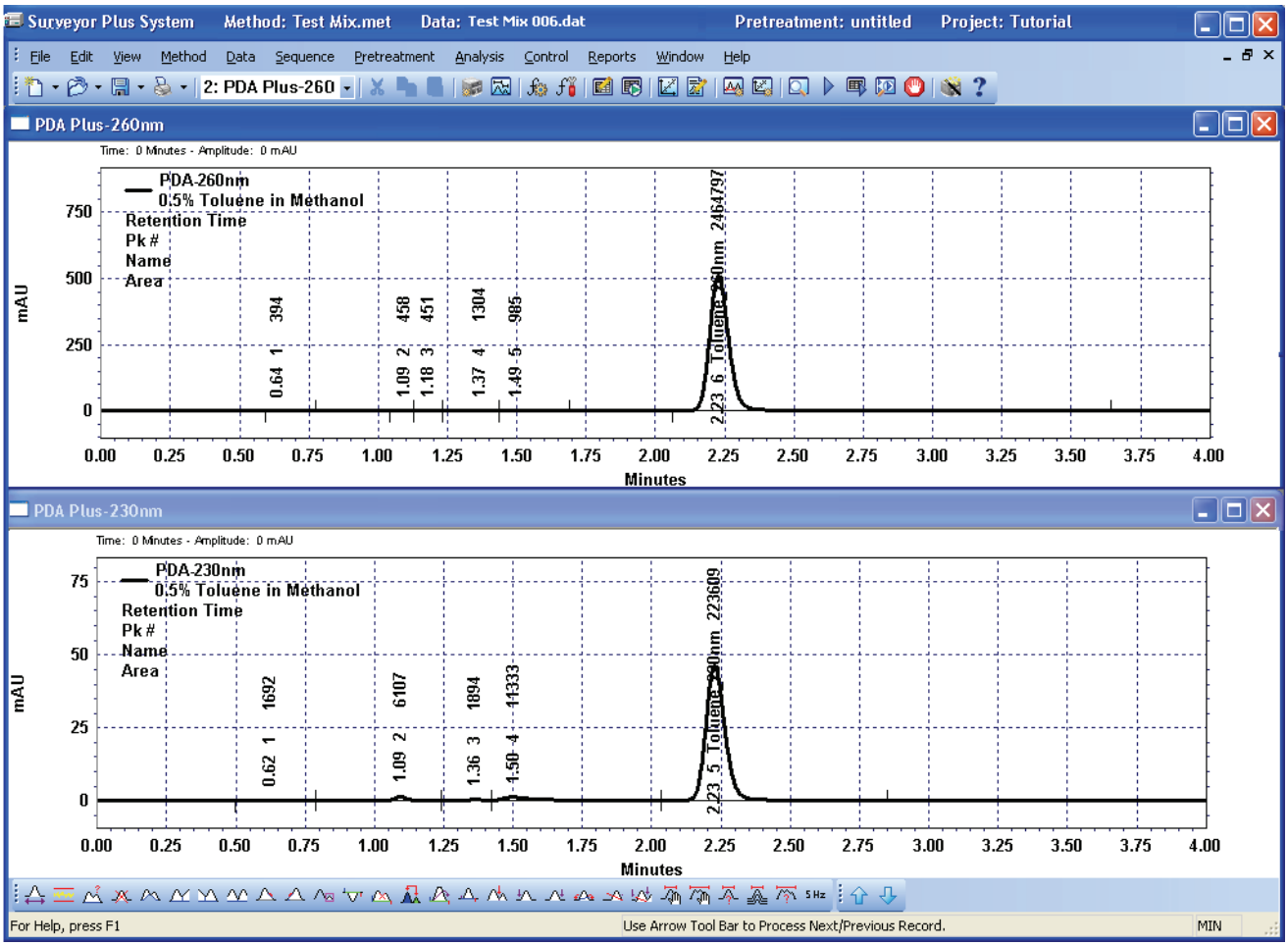
3. Click **Start**.

   The review process commences.

4. Click the blue up and down arrows in the bottom-right of the Instrument window to move through the rows of the sequence. See Figure 194.

5. After you review the last data file, click the green down arrow one more time to exit the sequence reprocessing mode.

**Figure 194.** Instrument window, showing the Chromatogram windows for both the 230 nm and the 260 nm analysis channels



# Diluting Samples with a Pretreatment Method

In this tutorial, you learn how to create and run a pretreatment method that dilutes samples ten-fold.

Pretreatment methods allow you to perform automated sample preparation operations. Pretreatment methods are created separately from acquisition methods. To perform a pretreatment method you must open it in the Single Run dialog box or add it to the sequence table. The Surveyor Autosampler performs the specified pretreatment method before it injects the sample. If you create a special type of "prep only" acquisition method, the autosampler does not make an injection.

**Contents**



## Creating a Pretreatment Method

In ChromQuest, pretreatment routines are created as separate methods. The Pretreatment dialog box allows you to create a multi-function pretreatment method in a table format, which can contain up to 512 rows. This section describes how to create a pretreatment method that dilutes samples ten-fold.

Create a pretreatment method that dilutes samples ten-fold by performing the following procedures:

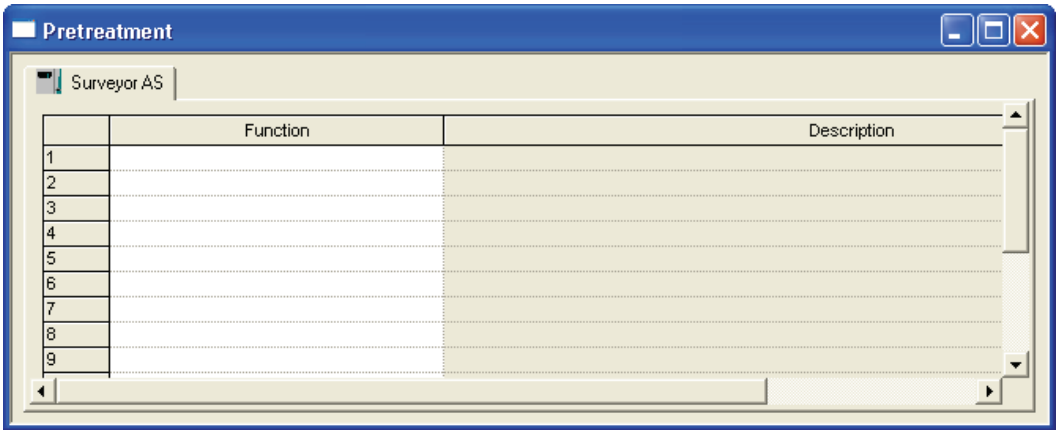
1. Opening the Pretreatment Window
2. Adding Functions to the Pretreatment Table
3. Saving the Pretreatment Method

## Opening the Pretreatment Window

❖ **To open the Pretreatment window from the Instrument window**

1. Choose **File > Pretreatment > New**.
2. Choose **Pretreatment > Edit** to open the Pretreatment window containing an empty pretreatment table. See Figure 195.

**Figure 195.** Pretreatment window, showing empty Pretreatment table




## Adding Functions to the Pretreatment Table

The Pretreatment window contains a table. Each line of the table contains an individual function that performs a particular task, such as transferring a specified volume of liquid from one vial location to another vial location. When you click the down-arrow in the Function column, a list of functions appears. Each function contains a set of parameters, such as transfer volume source vial location, and destination vial location. After you enter the parameters for a function, the Description column of the table lists the parameters that you selected.

To create a pretreatment table that dilutes samples ten-fold, perform the following procedures:

1. Adding the Transfer From Sample to Sample Function
2. Adding the Transfer From Reservoir To Sample Function
3. Adding the Wash Needle Function
4. Adding the Mix At Sample Function

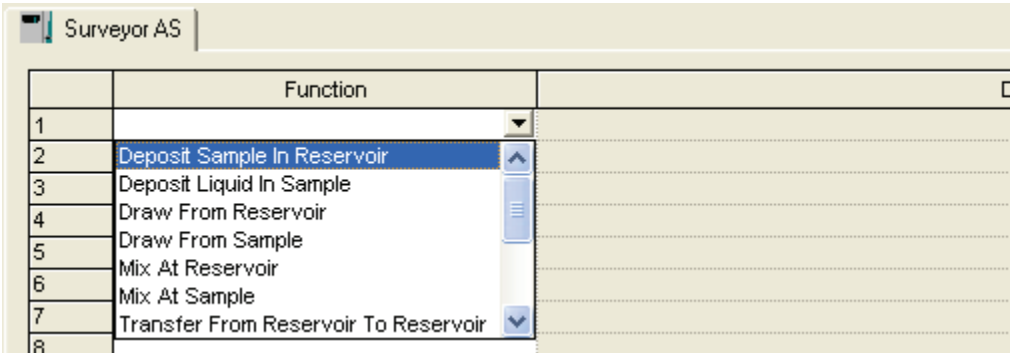
## Adding the Transfer From Sample to Sample Function

Your pretreatment method will transfer sample from the current vial location in the sequence table to the current + 1 vial location. For example, if the current vial location is A1, sample solution will be drawn from vial location A1 and transferred to vial location A2.

### ❖ To add the Transfer From Sample To Sample function

1. Click the first cell of the Function column to make the down-arrow appear.
2. Click the down-arrow in the Function column to open the list of pretreatment functions. See Figure 196.

   **Figure 196.** Function list




3. Select the **Transfer From Sample To Sample** function.

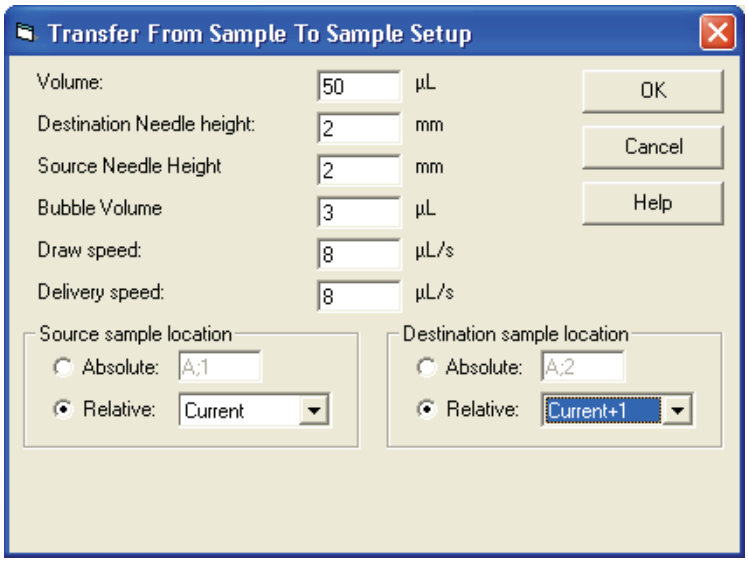
   ChromQuest opens a dialog box for the Transfer From Sample To Sample function.

4. Keep all parameters in the Transfer From Sample To Sample Setup dialog box set to the default settings except those that are listed in the following table and are shown in Figure 197.
5. Click **OK**.

| Parameter | Setting | Result |
|---|---|---|
| Volume | 50 | Specifies that 50 µL of sample will be withdrawn |
| Source Sample Location | | |
| • Source Sample Location | ◉ Relative | Turns on the Relative vial location option |
| • Relative List | Current | Specifies that the sample will be withdrawn from the current vial location in the sequence table |

| Parameter | Setting | Result |
|---|---|---|
| Destination Sample Location | | |
| • Destination Sample Location option | ◉ Relative | Turns on the Relative vial location option |
| • Relative List Item | Current + 1 | Specifies that the sample will be transferred to the current + 1 vial location in the sequence table |

**Figure 197.** Transfer From Sample To Sample function dialog box




## Adding the Transfer From Reservoir To Sample Function

To transfer more than 265 µL of liquid, you have to use the outer plunger of the syringe, which is less precise than the inner plunger. Therefore, to keep the operation in the inner plunger, your pretreatment method will transfer 225 µL of solvent from reservoir bottle 1 to the current + 1 vial location in the sequence table, twice, for a total of 450 µL of diluent.

### ❖ To add the Transfer From Reservoir To Sample Function

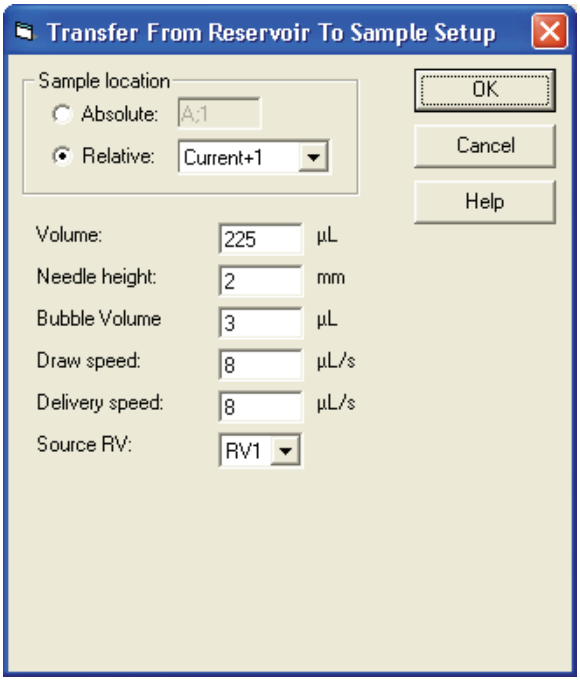
1. Click the second cell of the Function column, and then click the down-arrow to open the list of pretreatment functions.
2. Select the **Transfer From Reservoir To Sample** function.

   ChromQuest opens a dialog box for the Transfer From Reservoir To Sample function.
3. Keep all parameters in the Transfer From Reservoir To Sample Setup dialog box set to the default settings except those that are listed in the following table and are shown in Figure 198.

4. Click **OK.**
5. Click the third cell of the Function column, and then repeat step 2 through step 4 to add a second Transfer From Reservoir To Sample step.

| Parameter | Setting | Result |
|---|---|---|
| Volume | 225 | Specifies that 225 µL of solution will be withdrawn |
| Source RV | RV1 | Specifies that the solution will be drawn from reservoir vial 1 |
| Sample Location | | |
| • Sample Location option | ◉ Relative | Turns on the Relative vial location option |
| • Relative List Item | Current +1 | Specifies that the diluent drawn from the reservoir vial will be delivered to vial location current +1 |

**Figure 198.** Transfer From Reservoir To Sample Setup dialog box



## Adding the Wash Needle Function

After performing the Transfer From Sample To Sample function and the Transfer From Reservoir To Sample function, both of which used the inner plunger of the concentric syringe, the autosampler must home the plungers of the concentric syringe before it can perform the Mix At Sample function, which uses the outer plunger of the concentric syringe.

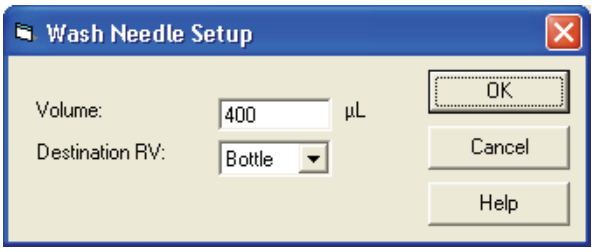
There are two functions that home the syringe: Wash Needle and Flush To Waste. For your pretreatment method, you will use the Wash Needle function to home the plungers of the concentric syringe.

### ❖ To add the Wash Needle function to the pretreatment table

1. Click the third cell of the Function column, and then click the down-arrow to open the list of pretreatment functions.
2. Select the **Wash Needle** function.

   The Wash Needle Setup dialog box appears.
3. Keep all parameters in the Wash Needle Setup dialog box set to the default settings, as shown in Figure 199.
4. Click **OK.**

**Figure 199.** Wash Needle Setup dialog box



## Adding the Mix At Sample Function

At this point, the vial in the current + 1 vial location contains 50 µL of the original 0.5% toluene in methanol sample solution and an additional 450 µL of methanol. Before you inject this solution, you want to mix it. The mixing function in ChromQuest aspirates liquid into the needle tubing, and then expunges it.

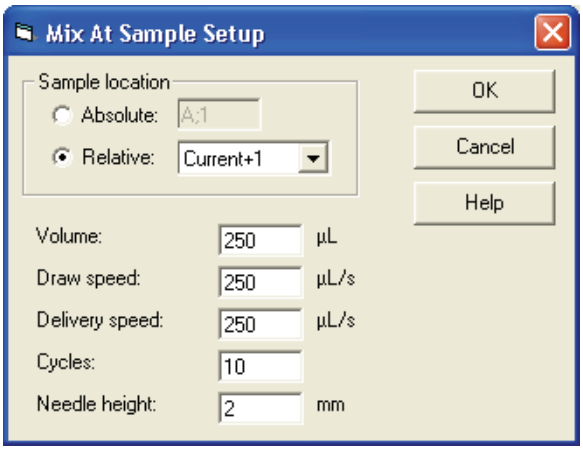
### ❖ To add the Mix At Sample function to your pretreatment method

1. Click the fourth cell of the Function column, and then click the down-arrow to open the list of pretreatment functions.
2. Select the **Mix At Sample** function.

   The Mix At Sample Setup dialog box appears.
3. Keep all parameters in the Mix At Sample Setup dialog box set to the default settings except those that are listed in the following table and are shown in Figure 200.
4. Click **OK**.

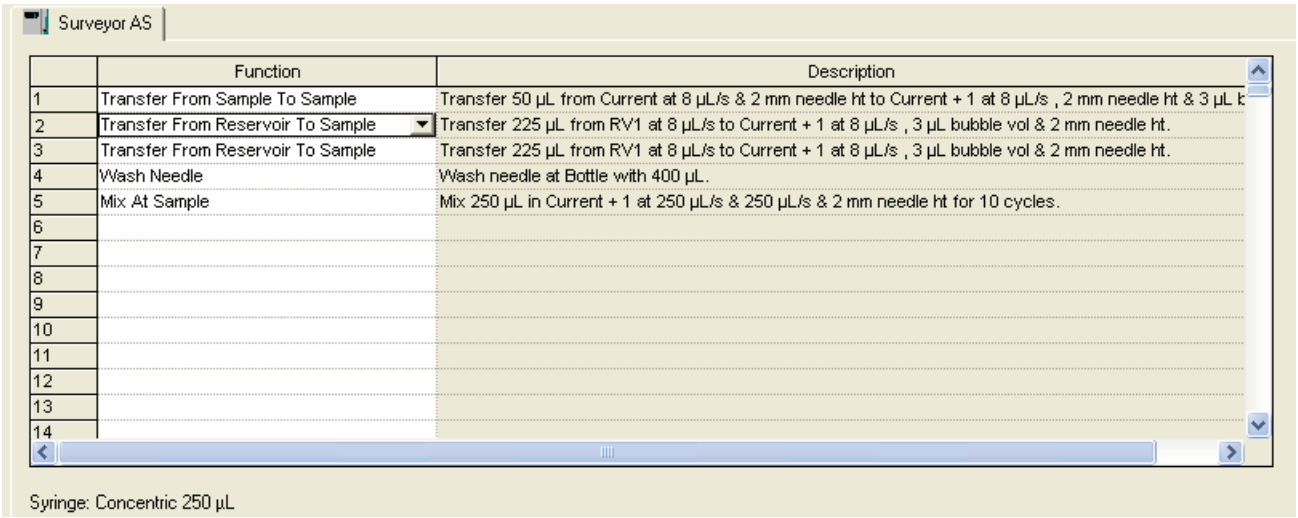
| Parameter | Setting | Result |
|---|---|---|
| Sample Location | | |
| • Sample Location Mode | ◉ Relative | Specifies that 50 µL of sample will be withdrawn |
| • Relative List Item | Current + 1 | Specifies that the sample will be transferred to the current + 1 vial location in the sequence table |
| Volume | 250 µL | Specifies that 250 µL of sample will be aspirated into the needle tubing, and then expunged back into the vial |
| Cycles | 10 | Specifies that the sample will be aspirated and expunged 10 times |

**Figure 200.** Mix At Sample Setup dialog box




You have entered the functions required to perform a ten-fold sample dilution. Verify that your pretreatment table matches the one shown in Figure 201. Then save your pretreatment method as described in the next topic Saving the Pretreatment Method.

**Figure 201.** Pretreatment table, displaying a Pretreatment method that performs a ten-fold dilution



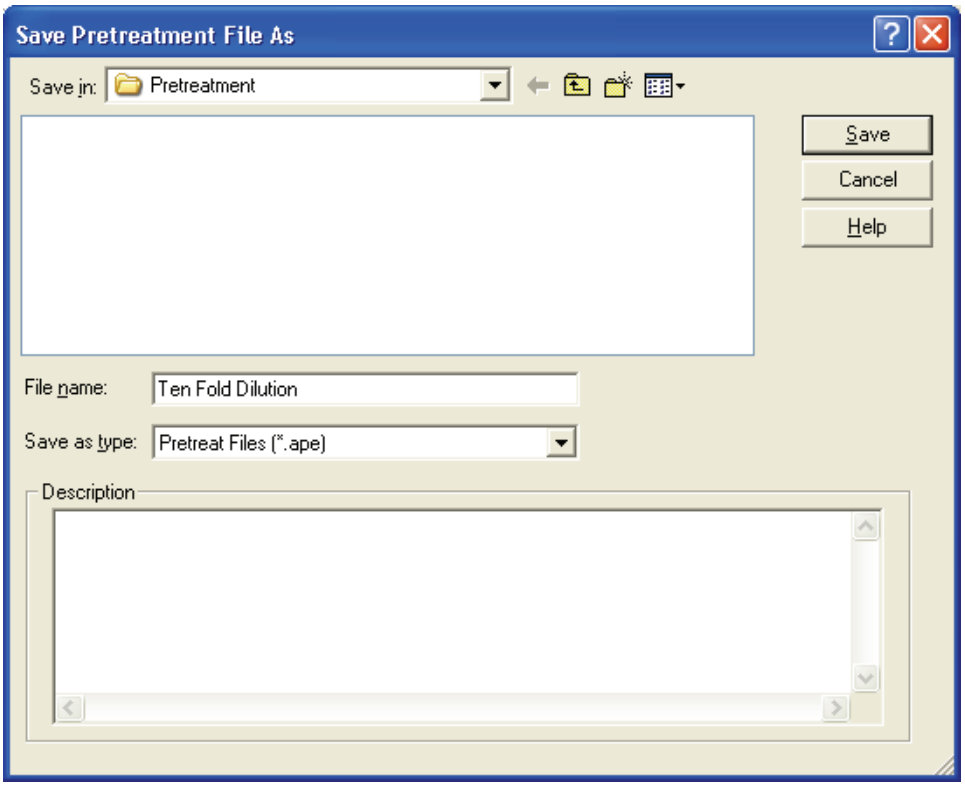
## Saving the Pretreatment Method

### ❖ To save the Pretreatment method

1. Choose **File > Pretreatment > Save As** to open the Save Pretreatment File As dialog box. See Figure 202.
2. Browse to the Pretreatment folder.
3. Type the name **Ten Fold Dilution** in the filename box.
4. Click **Save**.

   Pretreatment methods are saved with the file extension .ape.

**Figure 202.** Save Pretreatment File As dialog box



# Creating a Prep Only Method

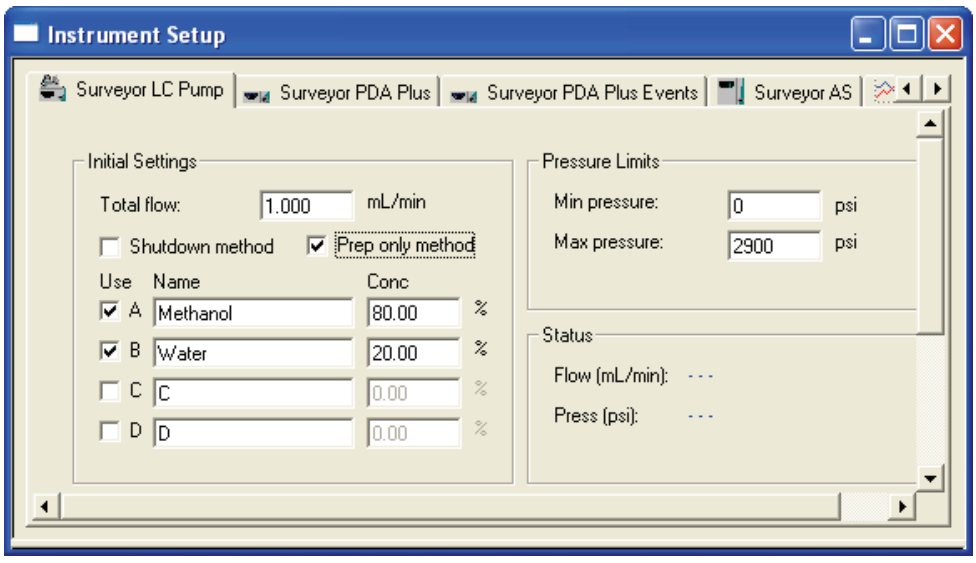
To create a method that can run without triggering an injection or data acquisition, you must select the Prep Only Method check box for every Surveyor module of your instrument.

In a "prep only method", the mobile phase conditions are maintained at the initial settings in the pump program. No injection and no data acquisition occurs. If the instrument setup page for the autosampler specifies timed events they are not performed.

### ❖ To create a prep only method

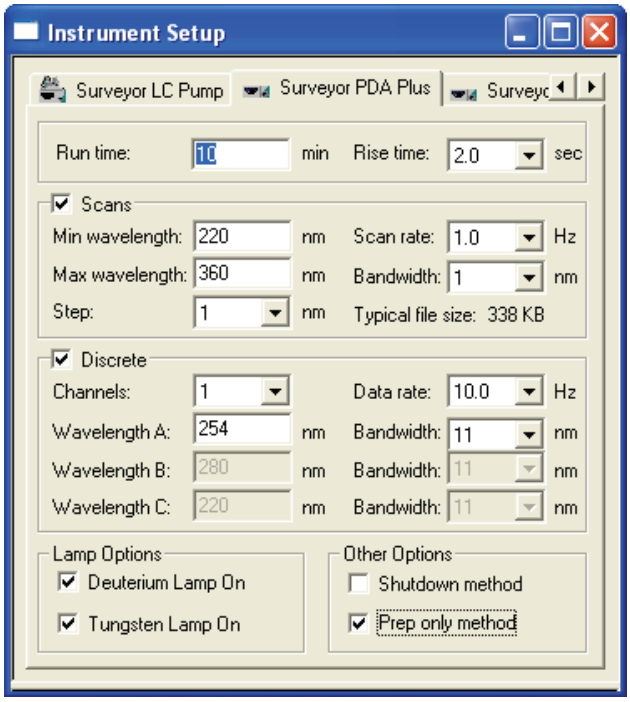
1. From the Instrument window, choose **File > Method > Open**.
2. From the Open Method File dialog box, select the method that you created while performing "Creating an Acquisition Method" on page 81 or a method that contains the pump program that you want to use.
3. From the Instrument window, choose **Method > Instrument Setup**.
4. Click the **Surveyor LC Pump** tab, and then select the **Prep only method** check box. See Figure 203.

**Figure 203.** Prep only method for the Surveyor LC Pump Plus



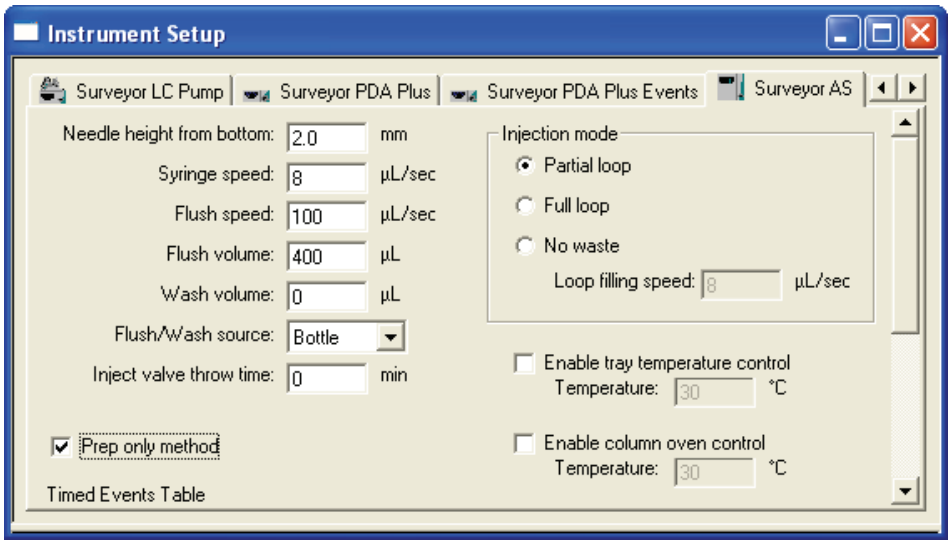
5. Click the tab for the detector, and then, select the **Prep only method** check box. See Figure 204.

**Figure 204.** Prep only method for the Surveyor PDA Detector




6. Click the **Surveyor AS** tab, and then select the **Prep only method** check box. See Figure 205.

**Figure 205.** Prep only method for the Surveyor Autosampler




7. Choose **File > Method > Save As** to display the Save Method File As dialog box.
8. Type **PrepOnly** in the File Name box, and then click **Save**.

# Using a Pretreatment Method

Pretreatment methods are separate from data acquisition methods. If you run a pretreatment method with a "prep only" method, the autosampler does not make an injection.

### ❖ To dilute the 0.5% toluene solution with methanol

1. Transfer the contents of an ampule of Autosampler Test Mix to a standard 1.8 mL vial.
2. Place the vial into vial location A1 of a standard tray.
3. Place an empty vial into vial location A2 of a standard tray.
4. Open the method named PrepOnly.met.
5. Click the **Single Run** button to open the Single Run Acquisition dialog box.
6. Keep all parameters in the Single Run Acquisition dialog box set to the default settings except those that are listed in the following table and shown in Figure 206.

   The active method PrepOnly.met is listed in the Method box.

| Parameter | Setting | Result |
|---|---|---|
| Run Information | | |
| • Method | PrepOnly.met | Downloads this method |
| Autosampler | | |
| • Use Program | ☑ | Enables the selection of a pretreatment method |
| • Program File | Ten Fold Dilution.ape | Specifies that the autosampler will perform the Ten Fold Dilution.ape pretreatment method |
| • Vial | A;1 | Specifies the sample location |
| • Injection Volume | 1 | No injection occurs. However this field requires a minimum value of 0.1 µL. |

7. Click **Run**.

   The run starts. The autosampler performs the pretreatment method, but does not make an injection.

**Figure 206.** Single Run Acquisition dialog box, showing the parameters for creating a diluted sample

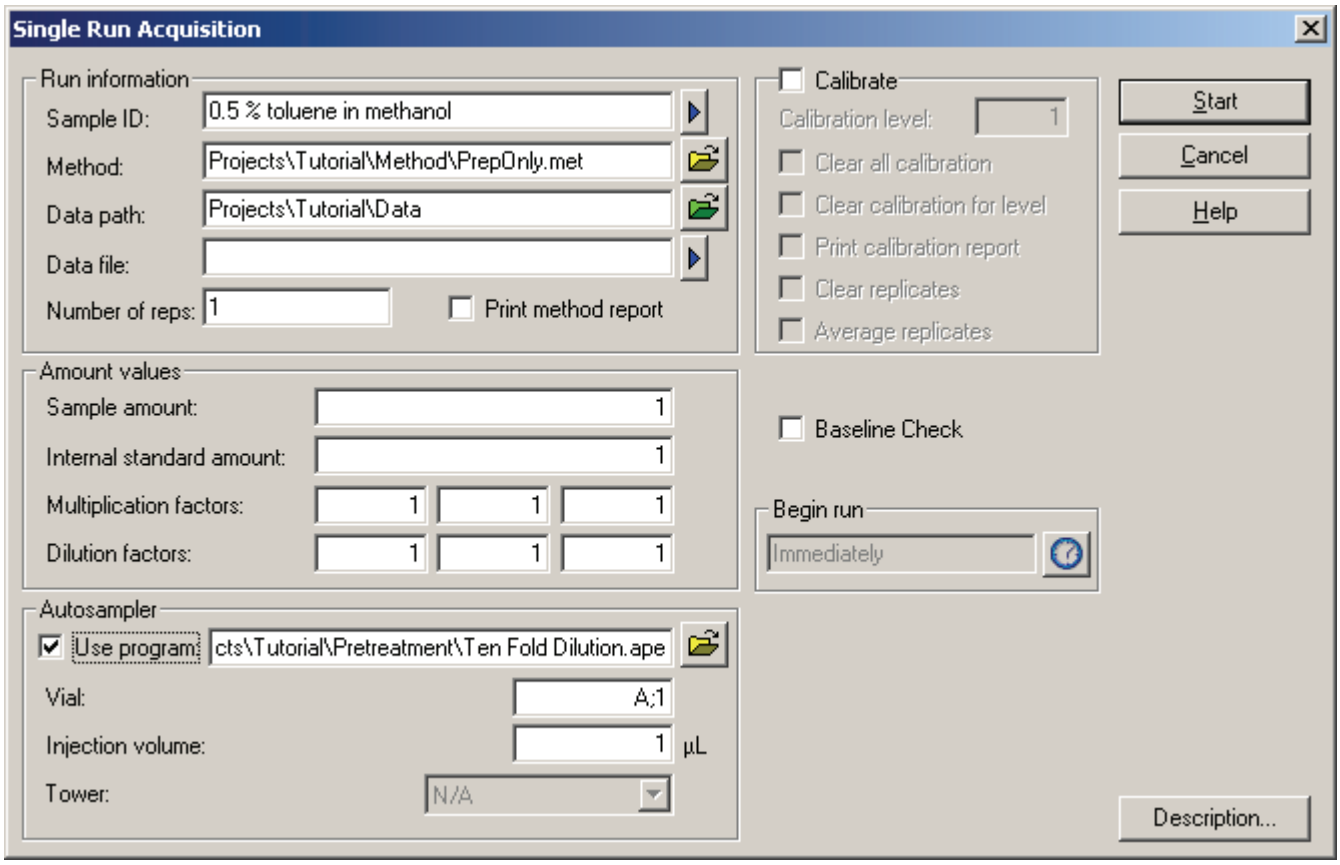



**Table 10.** Steps that occur during pretreatment method

| Step | Action |
|---|---|
| Transfer From Sample To Sample | The autosampler draws 50 µL of sample from vial location A1 (current), and then deposits the sample in vial location A2 (current + 1). |
| Transfer From Reservoir To Sample | The autosampler draws 225 µL of diluent from reservoir vial 1, and then deposits this solvent in vial location A2 (current + 1). |
| Transfer From Reservoir To Sample | The autosampler draws 225 µL of diluent from reservoir vial 1, and then deposits this solvent in vial location A2 (current + 1). |
| Wash Needle | The needle moves to the wash station where it expels 400 µL of solvent from the wash bottle. The autosampler initializes the concentric syringe. |
| Mix At Sample | The needle moves to vial location A2 where it aspirates and expels 250 µL of sample solution 10 times. |

### ❖ To inject the diluted sample

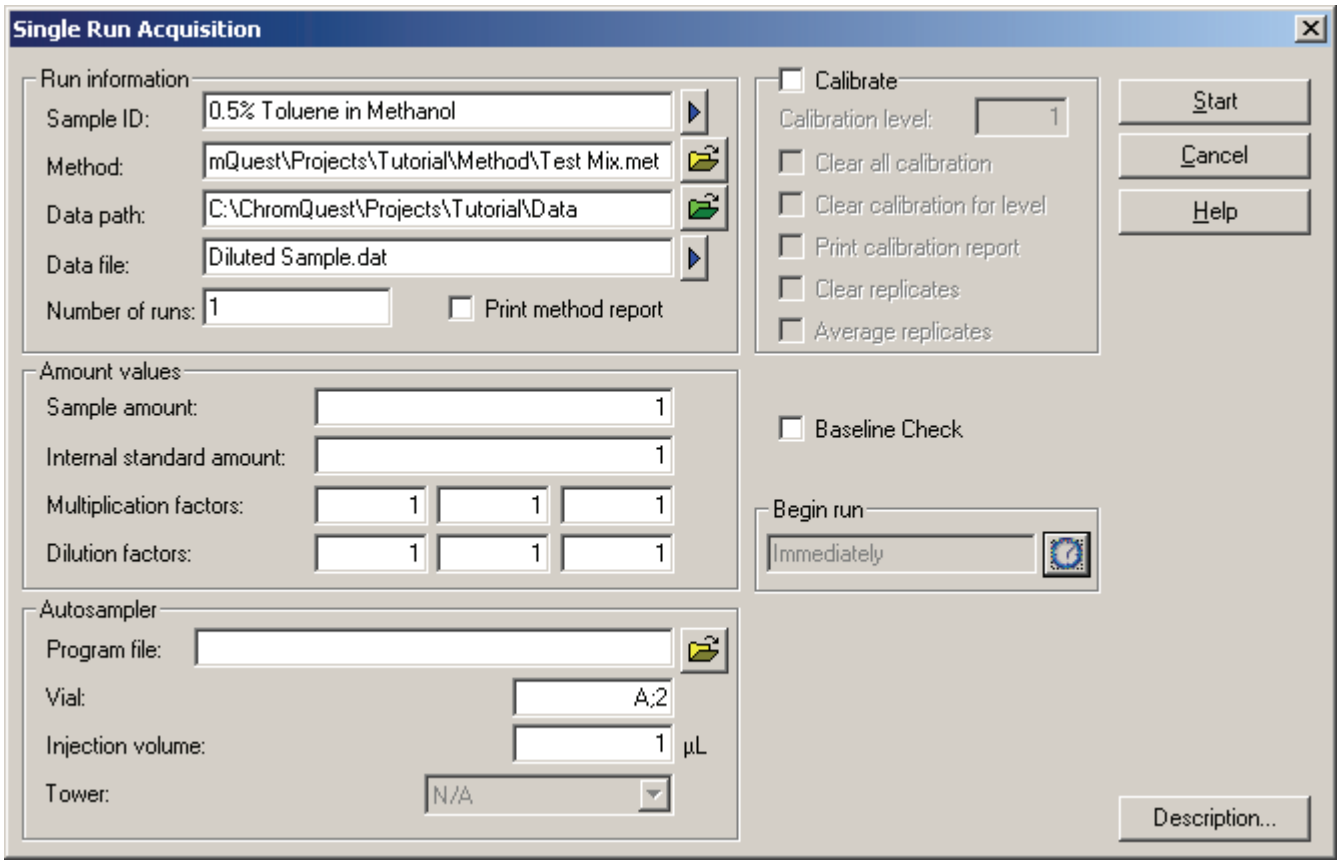
1. Click the **Single Run** button to open the Single Run Acquisition dialog box.
2. Keep all parameters in the Single Run Acquisition dialog box set to the default settings except those that are listed in the following table and shown in Figure 207.

| Parameter | Setting | Result |
|---|---|---|
| Run Information | | |
| • Sample ID | 0.05% Toluene in Methanol | Gives the data file a sample ID, which can be used in the search feature within ChromQuest |
| • Method | Test Mix.met | Downloads this method |
| • Data Path | Drive:\ChromQuest\Projects\Tutorial\Data | Stores the data file in this folder |
| • Data File | Diluted Sample.dat | The data file will be named Diluted Sample.dat |
| • Print Method Report | ☑ | Specifies that the custom report will be printed after the data file is acquired |
| Autosampler | | |
| • Use Program | ☐ | Specifies that the autosampler will not perform a pretreatment method |
| • Vial | A;2 | Specifies that the autosampler withdraws sample from vial location A2 |
| • Injection Volume | If you are using a 5 cm LightPipe flow cell, enter a value of 1.<br><br>If you are using a standard 1 cm flow cell, enter a value of 5. | Specifies that the autosampler will inject 1 µL or 5 µL, respectively, of sample into the mobile phase stream |

3. Click **Run**.

   The run starts. The autosampler does not perform a pretreatment method because you have removed the method from the Program File box. Compare the custom reports for data files Preliminary Run.dat and Diluted Sample.dat. The area of the toluene peak in the diluted sample will be approximately one-tenth the area for the toluene in the original sample.

**Figure 207.** Single Run Acquisition dialog box, showing parameters for injecting the diluted sample



# Pretreatment Rules

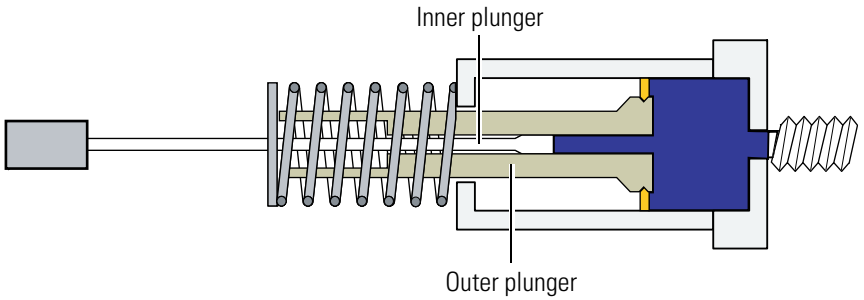
The maximum volume that can be deposited, drawn, or transferred during a pretreatment step depends on the syringe type, the function requested, and the previous step in the pretreatment method.

For the concentric syringes (see Figure 208), the manner in which the various pretreatment functions can be linked together is affected by whether the function takes place in only the large bore of the syringe, only the small bore of the syringe, or in either bore of the syringe. The pretreatment functions can be divided into three groups depending on whether they use only the small bore of the syringe, only the large bore of the syringe, or either bore of the syringe. See Table 11.

**Table 11.** Functions Grouped According to Bore Usage

| Small Bore Only | Large Bore Only | Small or Large Bore |
|---|---|---|
| Draw from Sample | Mix at Sample | Draw from Reservoir |
| Transfer from Sample to Sample | Mix at Reservoir | Deposit Liquid in Sample |
| Transfer from Sample to Reservoir | Wash Needle | Deposit Sample in Reservoir |
| | Flush to Waste | Transfer from Reservoir to Sample |
| | | Transfer from Reservoir to Reservoir |

**Figure 208.** Concentric syringe, showing inner and outer plungers



If you are using a concentric syringe, the arrangement of the functions in the pretreatment table is restricted by the following four rules:

**Note** You cannot save a method that does not conform to these rules.

1. For functions that are performed with the small bore of the concentric syringe, the maximum volume (sample + air bubble) that you can draw, deposit, or transfer is limited to the nominal size of the syringe.
2. Functions performed with the large bore of the concentric syringe, the maximum volume (sample + air bubble) that you can draw, deposit, or transfer is limited to 500 µL. The volume range for the Wash Needle and Flush to Waste functions is 100 to 6000 µL. For flush or wash volumes greater than 539 µL, multiple draws are performed.
3. If a function can be performed from either the small or the large bore:
   - The concentric syringe uses the small bore if the requested volume (liquid + air bubble) plus any volume left in the needle tubing from a previous step is less than the nominal syringe size.
   - The syringe uses the large bore if the requested volume (liquid + air bubble) is greater than the nominal syringe size.
   - If the previous step started in the small bore, and the requested volume plus any volume left in the needle tubing from the previous step is greater than the nominal syringe size, the step will not be allowed. Crossover between bores is not allowed.
4. You cannot add a function that requires the use of the large syringe bore immediately following a function that requires the use of the small syringe bore. To switch from the small bore to the large bore of the syringe, you must insert a Flush to Waste step or a Wash Needle step. These functions initialize the plungers of the syringe.

# Calibration Procedures

**Contents**



The modules of your Surveyor Plus instrument are factory calibrated. The PDA detector is tested for linearity, noise, and drift. The LC pump is calibrated to produce an accurate flow rate while pumping water at 1 mL/min. Its ability to accurately proportion binary mobile phases is also tested at a flow rate of 1 mL/min. The column oven and the tray temperature zones of the autosampler are calibrated at 30 °C. The positioning of its XYZ arm mechanism is calibrated for the carrier trays supplied by Thermo Fisher Scientific.

Because of the sensitivity of its optical bench, it is best to recalibrate the Surveyor PDA Plus Detector after you install it, each time you move it, change its flow cell, or replace either of the lamps. In addition, you might need increase or decrease the amount of incident light reaching the diode array by adjusting the attenuators. Over time, the performance of the lamps deteriorates. Using the built-in holmium oxide filter, you can monitor the performance of the lamps and establish an appropriate lamp replacement schedule.

Perform the validation procedure for the Surveyor RI Plus Detector after you install it and periodically thereafter. The validation procedure for the RI detector verifies the full-scale range of the detector based on the detector's response to a 0.35% by weight sucrose / water solution. In addition, the software contains procedures for verifying the analog output signals so that you can use a chart recorder or an integrator to collect data.

The Surveyor Autosampler Plus does not require calibration upon arrival at its shipping destination. However, if you plan to use custom vials or custom microplates, you must perform the "Bottom Distance Calibration" on page 255 to determine their depth. If problems occur with the column oven, the tray temperature control, or the arm positioning of autosampler, contact a Thermo Fisher Scientific service representative. Procedures for calibrating the temperature of the column oven and tray compartments are included in this appendix for those users who prefer to maintain their own instruments.

Like the autosampler, the Surveyor LC Pump Plus does not require calibration upon arrival at its shipping destination. However, over time, the pressure readings from its built-in pressure transducer can drift, thereby requiring re-zeroing.

# Verifying the Performance of the PDA Detector

This section contains the following procedures:

- Adjusting the Light Intensity
- Performing a Wavelength Calibration
- Performing an Array Calibration
- Checking the Status of the Lamps

## Adjusting the Light Intensity

The Surveyor PDA Plus Detector has two attenuators that control the light output from the lamps. During the lifetime of the Surveyor PDA Plus Detector it might be necessary to adjust the attenuators to increase or decrease the amount of light falling onto the array.

Decreasing light output increases baseline noise. Increasing light output can cause saturation of the diode array. If the array is saturated the response from the Surveyor PDA Plus Detector will be a flat baseline.

The attenuators require adjustment when either lamp is replaced or when the flowcell is replaced. When any of these items are replaced, check the light intensity by following the Operational Verification procedure and adjust the attenuators to provide light intensities in the specified operating ranges.

**Note** Before you adjust the attenuators, replace the column with a flow restrictor, and set the pump to deliver HPLC-grade water at a flow rate of 1 mL/min through the flowcell.

To adjust the light output from the lamps, perform the following procedures:

1. Turning on the Lamps
2. Setting the Parameters for the Spectral Display
3. Determining the Diode of Maximum Intensity for the UV Range
4. Determining the Diode of Maximum Intensity for the Visible Range
5. Setting the Discrete Channel Displays
6. Adjusting the Attenuators

## Turning on the Lamps

### ❖ To turn on the lamps

1. From the Windows XP taskbar, choose **Start > All Programs > Chromatography > ChromQuest** to open ChromQuest.
2. In the ChromQuest Main Menu, double-click the icon for your instrument to open the online Instrument window.
3. From the menu bar, choose **Control > Instrument Status** to open the Instrument Status window.
4. Click the **Surveyor PDA Plus** tab to open the Surveyor PDA Plus Instrument Status page.
5. Note the usage hours for each lamp.
6. Verify that both lamps are On. If they are not On, click **On** for both lamps.

## Setting the Parameters for the Spectral Display

### ❖ To set the parameters for the spectral display of the lamp intensities

1. From the Instrument Status page for the Surveyor PDA Plus, click **Diagnostics**.
2. Click the **Control** tab.
3. Under Mode, click the **Intensity** option.
4. Click **Default**, and then verify that the following parameters are specified:

   Start = 2
   End = 511
   Step = 1
   Bandwidth = 1
   Scan rate = 1

5. Click **Load To Detector**.

   A dialog box containing the following message “Method Has Been Downloaded” appears.

6. Click **OK**.

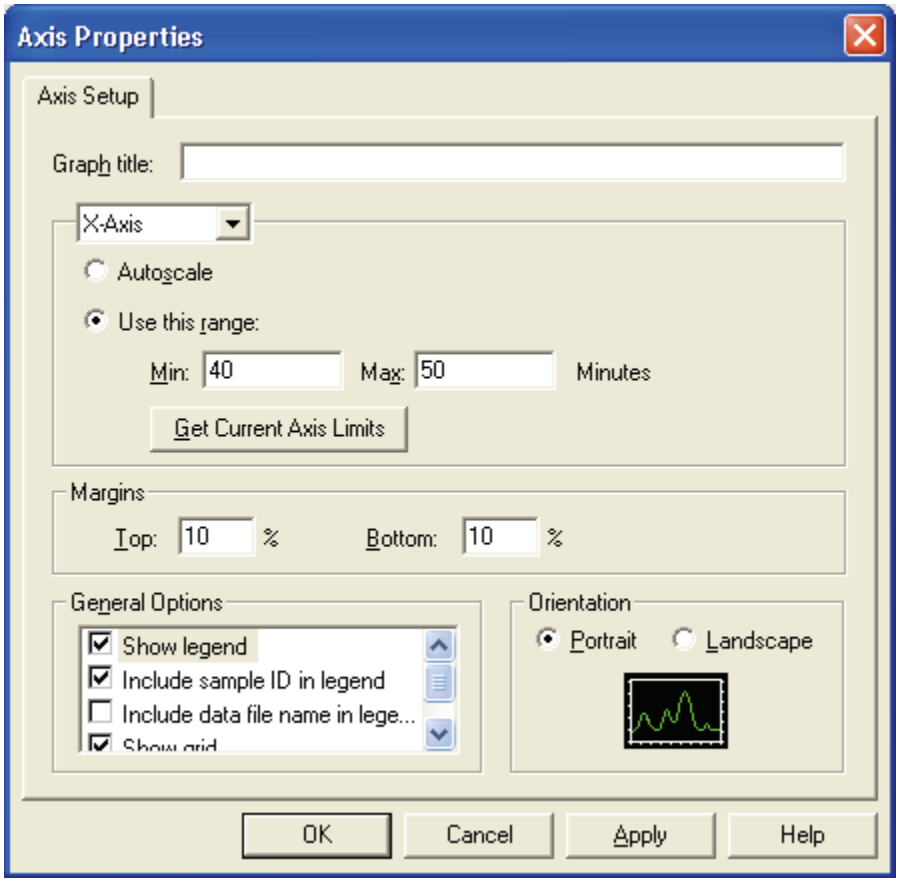
## Determining the Diode of Maximum Intensity for the UV Range

❖ **To determine the diode of maximum intensity for the deuterium lamp (UV range)**

1. Click the **Display** tab to open the Display page.
2. Click **Start** located in the upper right corner of the Display page.
3. Right-click in the top pane containing the intensity spectrum and choose **Axis Setup**.

   The Axis Properties dialog box, shown in Figure 209, appears.

   **Figure 209.** Axis Properties dialog box




4. In the Axis Properties dialog box, do the following:
   a. Select **X-axis** from the list.
   b. Click the **Use this range** option.
   c. Type a Min value of **30** and a Max value of **50** for the diode range.
   d. Click **OK** to close the dialog box and update the scaling of the Spectrum view.
5. From the spectrum displayed, determine and record the pixel of maximum intensity within the 30 to 50 diode range. This is the diode of maximum output for the deuterium lamp.

## Determining the Diode of Maximum Intensity for the Visible Range

### ❖ To determine the diode of maximum intensity for the visible range

1. Open the Axis Setup dialog box.
2. Type a a Min value of **400** and a Max value of **500** for the diode range.
3. Click **OK** to close the dialog box and update the scaling of the Spectrum view.
4. In the spectrum displayed, determine and record the pixel of maximum intensity within the 400 to 500 diode range. This is the diode of maximum output for the tungsten lamp.
5. Turn off the data stream by clicking **Off** for Status and **Stop** for Data.

## Setting the Discrete Channel Displays

### ❖ To set the discrete channel displays

1. Click the Control tab to open the Control page.
2. Type the value for the diode of maximum intensity for the deuterium lamp in the Channel A box.
3. Type the value for the diode of maximum intensity for the tungsten lamp in the Channel C box.
4. Click **Load To Detector**.

   A dialog box containing the following message “Method Has Been Downloaded” appears.
5. Click **OK**.

## Adjusting the Attenuators

### ❖ To adjust the attenuators

1. Open the front doors of the detector.
2. Unscrew the captive screw, and then remove the flowcell cover.

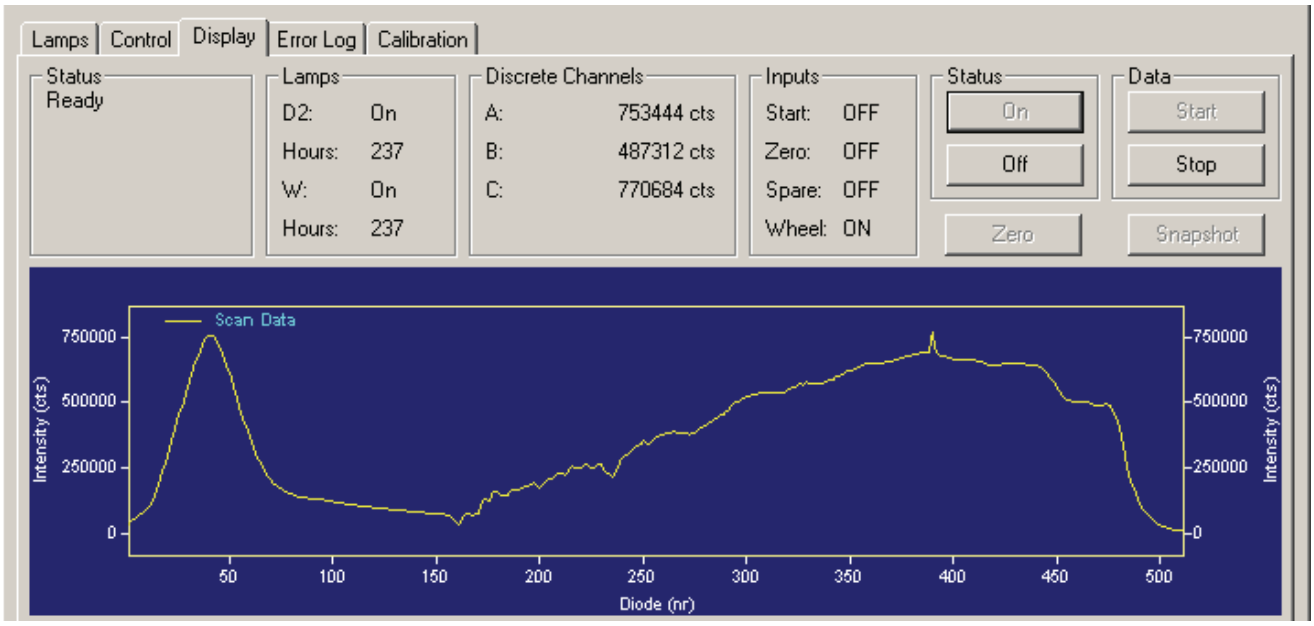
   The attenuators are located on the right side of the front panel. There are two black tabs attached to the attenuators for manual adjustments. See Figure 210.

   The left tab is the deuterium lamp attenuator and the right tab is the tungsten lamp attenuator. Pushing the attenuator tab up increases light output and pulling the tab down decreases light output.

**Figure 210.** Front view of the detector, showing the attenuators


3. Click the **Display** tab to open the Display page (see Figure 211).
4. Adjust the attenuators:
   a. Adjust the attenuator with the left tab on the PDA (UV attenuation) to achieve a Channel A value of between 750000 and 775,000 intensity counts.
   b. Adjust the attenuator with the right tab (Visible attenuation) to achieve a Channel C value of between 750000 and 775000 intensity counts.
5. After you finish adjusting the attenuators, replace the flowcell access cover and close the front doors of the detector.

**Figure 211.** Diagnostics dialog box – Display page, showing adjustment of Discrete Channels A and B



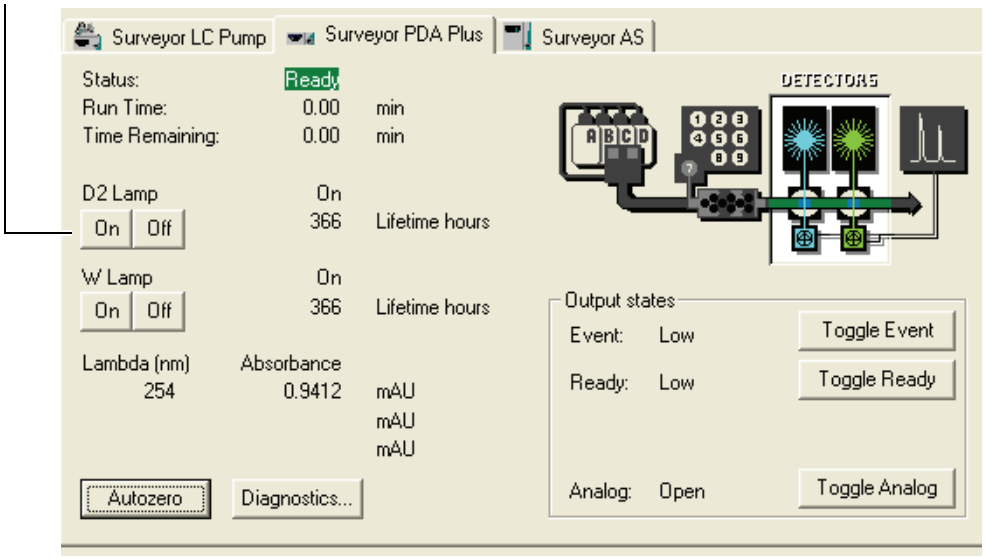

## Performing a Wavelength Calibration

The alignment of the spectrum on the diode array is dependent upon the physical alignment of various components of the optical bench. The alignment can become offset if the detector is sharply jolted, for example, in shipping. ChromQuest allows you to determine the wavelength accuracy of the optical bench and to mathematically correct for any alignment changes, if necessary. We recommend that you perform a wavelength calibration whenever you move the detector or download new firmware.

### ❖ To perform a wavelength calibration

1. Prepare the detector for a wavelength calibration:
   a. Replace the column with a flow restrictor.
   b. Pump HPLC- grade water or HPLC-grade methanol through the flow cell for at least one hour.
   c. Turn on both lamps by clicking the On buttons for the lamps in the Surveyor PDA Plus page of the Instrument Status window.
   d. Wait 1 hour for the D2 lamp to equilibrate.

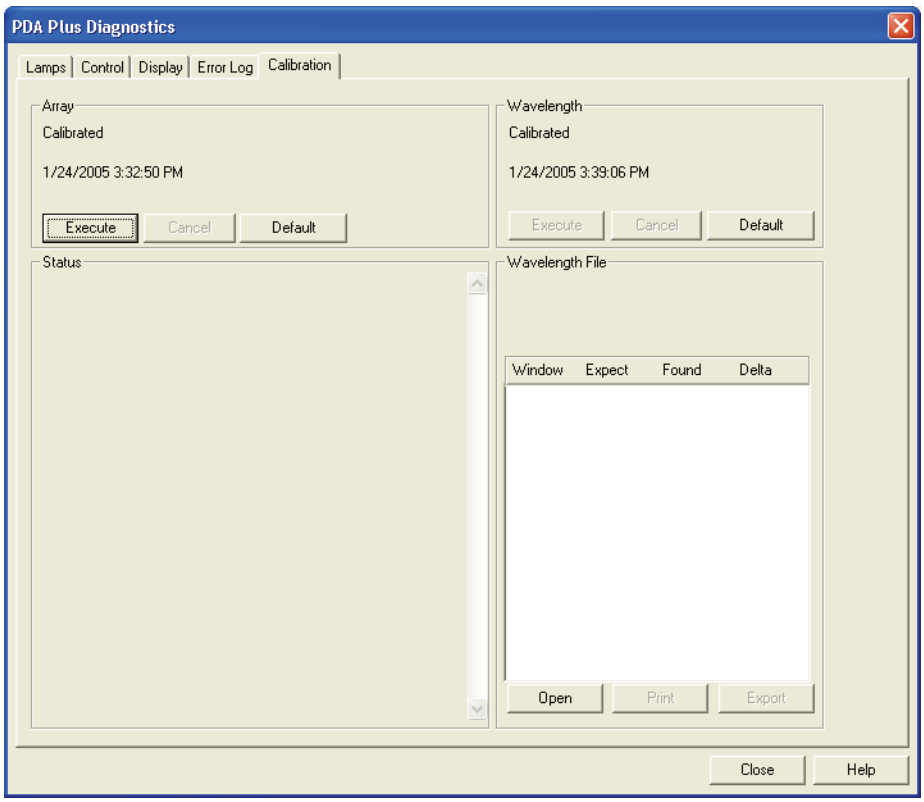
**Figure 212.** ChromQuest Instrument Status dialog box, showing the Surveyor PDA Plus page




2. In the Surveyor PDA Plus Instrument Status page, click **Diagnostics** to open the Surveyor PDA Plus Diagnostics dialog box.

3. Click the Calibration tab to open the Calibration page. See Figure 213.

**Figure 213.** PDA Plus Diagnostics dialog box – Calibration page



4. Open a Wavelength Calibration file:

   a. Under Wavelength File, click **Open** to open the Select Wavelength Calibration dialog box.

   b. In the Select Wavelength Calibration dialog box, shown in Figure 214, select an appropriate wavelength calibration file from the list. An appropriate wavelength file should include the range of wavelengths that you use under normal operation conditions.

   c. Click **Open**.

> **Note** ChromQuest has four calibration files to choose from. The HolmiumUV file contains five wavelengths in the UV region while the other files, such as Holmium12, use sets of wavelengths from both the UV and Visible wavelength regions. The holmium oxide absorbance maxima are selected from a spectrum published in "Holmium Oxide Solution Wavelength Standard from 240 to 640 nm - SRM 2034 (NIST Special Publication 260-54)".

The holmium oxide bands of the selected file are displayed in the Wavelength File area, as shown in Figure 215.

**Figure 214.** Select Wavelength Calibration dialog box

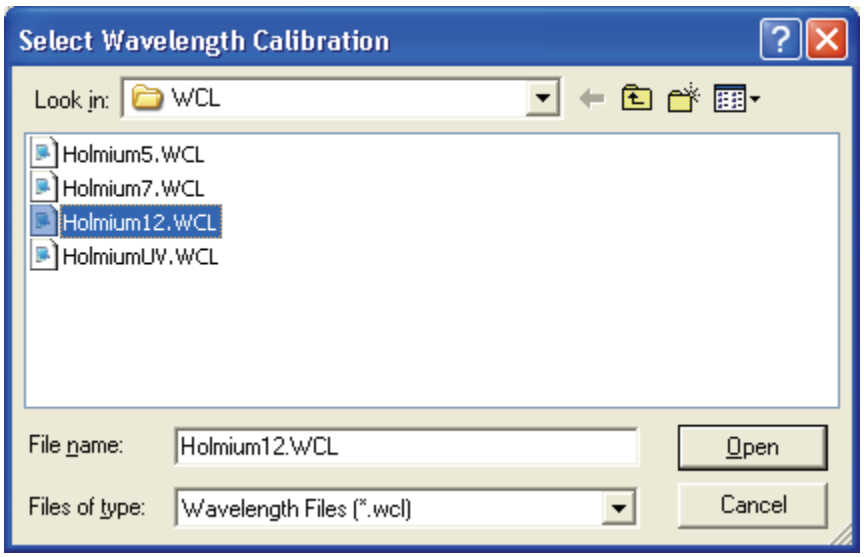
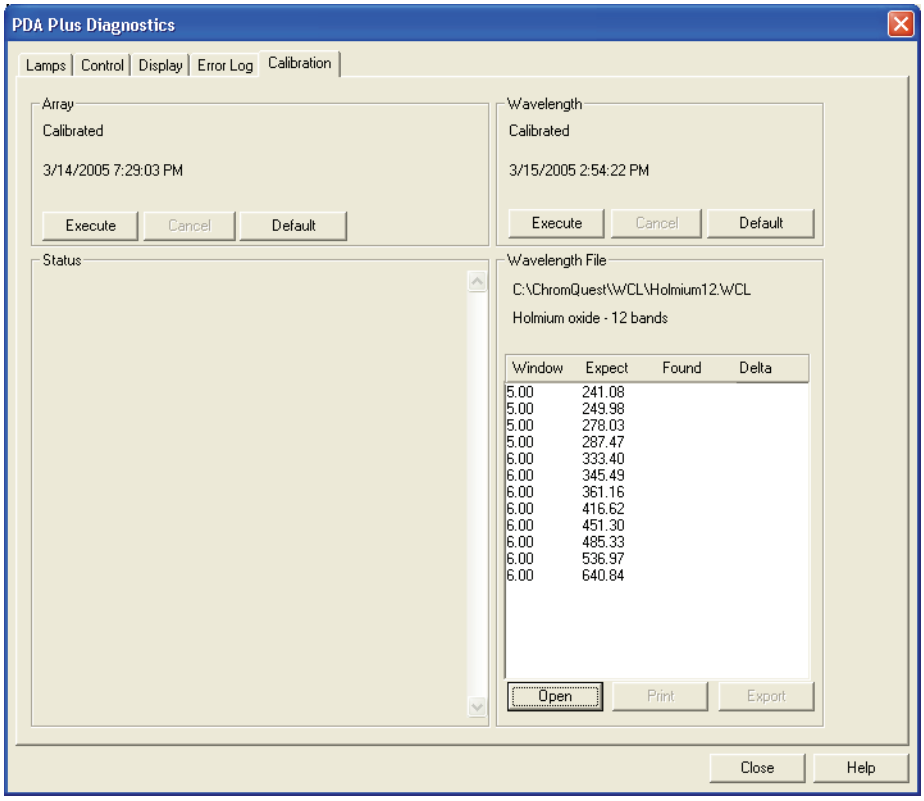


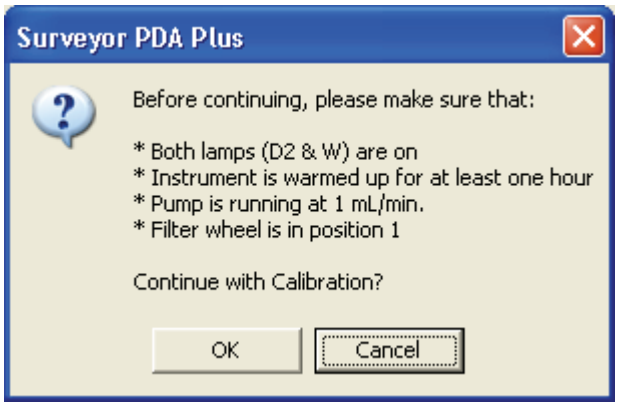
**Figure 215.** Diagnostics dialog box – Calibration page, showing the Holmium12 Wavelength Calibration File selected




5. In the Wavelength area, click **Execute**.

   A message box opens to remind you of all of the required preconditions. See Figure 216.

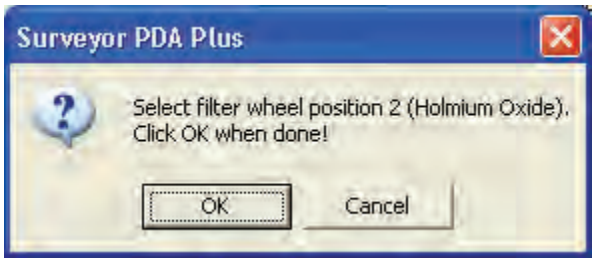
**Figure 216.** Calibration Preconditions message box



6. If you have met the preconditions, click **OK** in the message box.

   ChromQuest collects a background spectrum, which is used to remove the absorbance contribution of the mobile phase. When the background collection is complete, the message box, shown in Figure 217, appears.

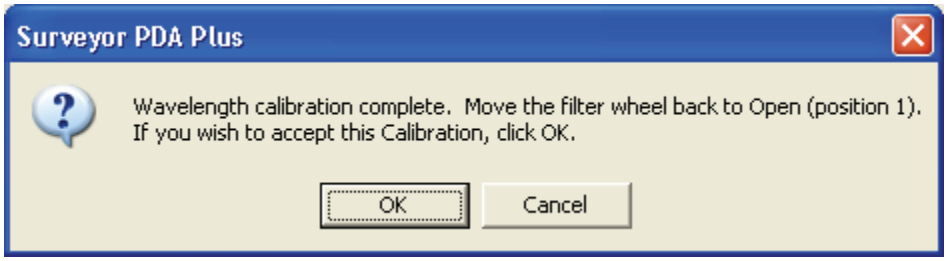
   **Figure 217.** Calibration message box




7. Move the filter wheel to position 2 as directed, and then click **OK** in the message box.

   The detector takes a holmium oxide scan, performs iterative calculations while applying the rise time and bandwidth filters, and then displays a new message box. See Figure 218.

   **Figure 218.** Calibration Complete message box




8. Move the filter wheel back to position 1, and then click **OK** to close the message box and view the results.

9. Check the delta values in the Wavelength File area (see Figure 219).

   a. If the delta values are not within the range of ±1 nm, repeat the wavelength calibration procedure for verification.

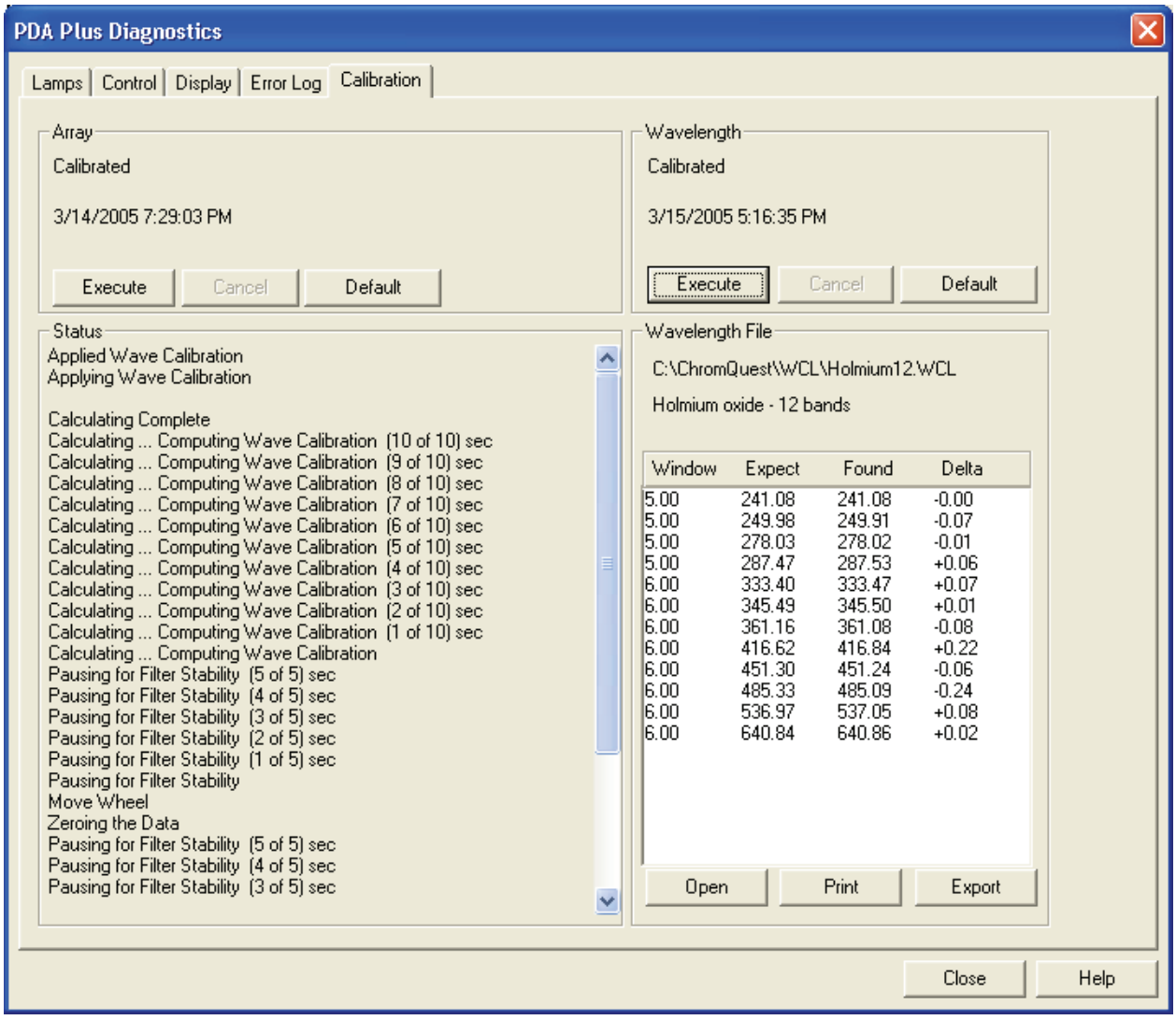
   b. If, after applying a new calibration, the delta values are still not within the range of ±1 nm, call your Thermo Fisher Scientific service representative for assistance.

10. If your data system PC is connected to a printer, click **Print** to print a hardcopy report. To store the results, click **Export**.

The date and time of calibration are displayed and are stored in memory.

**Tip** You can click **Cancel** in any of the Calibration dialog boxes at any time to abort the calibration process.

**Figure 219.** Calibration dialog box, showing acceptable Delta values



## Performing an Array Calibration

The function of the array calibration is to measure and correct for the dark current produced by the diodes of the photodiode array. The dark current is the small amount of background signal that is produced by the diodes of the array even when both lamps are turned off. Typical dark current values range from 1500 to 3000 counts.

Because the environmental conditions of your laboratory can cause the dark current of the diode array to increase over time, we recommend that you perform an array calibration (dark current) after 100 hours of use or monthly, whichever comes first; whenever a significant temperature change occurs; after you move the detector; after you replace the lamps; and after you download a new firmware file.

Because the dark current produced by the diodes rises as the temperature within the detector rises, it is important to warm up the lamps for 1 hour before you perform a dark current calibration. Warming up the lamps for 1 hour allows your detector to equilibrate to its normal operating temperature.

ChromQuest briefly turns the lamps off as it performs the dark current calibration routine. After it completes the dark current calibration routine, ChromQuest turns the lamps back on.

**Note** The dark current calibration program will not run when data collection is enabled on the Display page.

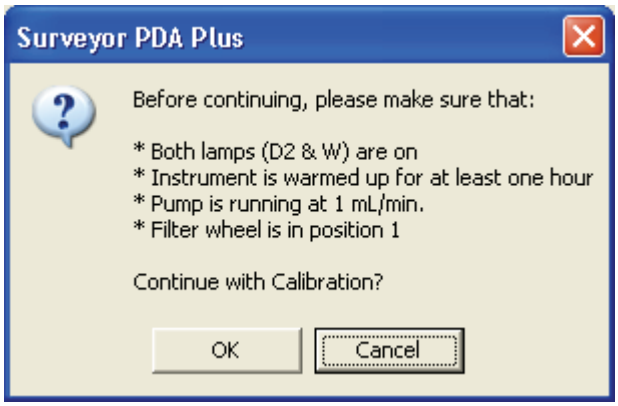
### ❖ To perform a dark current calibration of the diode array

1. Pump HPLC grade water or methanol at 1 mL/min through the flow cell for at least one hour.
2. Place the filter wheel of the Surveyor PDA Plus Detector in position 1 (Open).
3. Turn on both lamps and wait 1 hour for the $D_2$ lamp to equilibrate:
   a. From the online Instrument window, choose **Control > Instrument Status** to display the Instrument Status window.
   b. Click the Surveyor PDA Plus tab to display the Surveyor PDA Plus page. See Figure 212.
   c. Click the D2 (deuterium) Lamp On button and the W (tungsten) Lamp On button.

      This allows the detector to reach its normal operating temperature.
4. Click **Diagnostics** to open the Surveyor PDA Plus Diagnostics dialog box. Then click the Calibration tab to display the Calibration page. See Figure 220.
5. In the Array area, click **Execute**.

   A message box opens to remind you of the necessary preconditions. See Figure 220.

**Figure 220.** Calibration Preconditions message box




6. If you have met the preconditions, click **OK**.

   The status of the calibration procedure is displayed by the Status readback area on the Calibration page. During the dark current calibration, intensity scans are collected with both lamps Off. After the last event is completed, the lamps are turned back On.

7. Click **OK** to finish the calibration.

   The date and time of calibration are displayed and are stored in memory.

## Checking the Status of the Lamps

The performance of the deuterium lamp degrades over time, whereas the tungsten lamp tends to fail abruptly. As the performance of the deuterium lamp degrades, you might notice a decrease in the signal to noise ratio for the UV range. Replace the deuterium lamp after 1000 h of usage or as needed. Replace the tungsten lamp after 2500 h of usage or as needed.

You can monitor the status of the lamps from the Diagnostics dialog box – Lamps page.

This topic contains the following procedures:

- Viewing an Intensity Scan of Both Lamps Together
- Viewing an Intensity Scan of the Deuterium Lamp
- Viewing an Intensity Scan of the Tungsten Lamp
- Recording the Performance of the Lamps

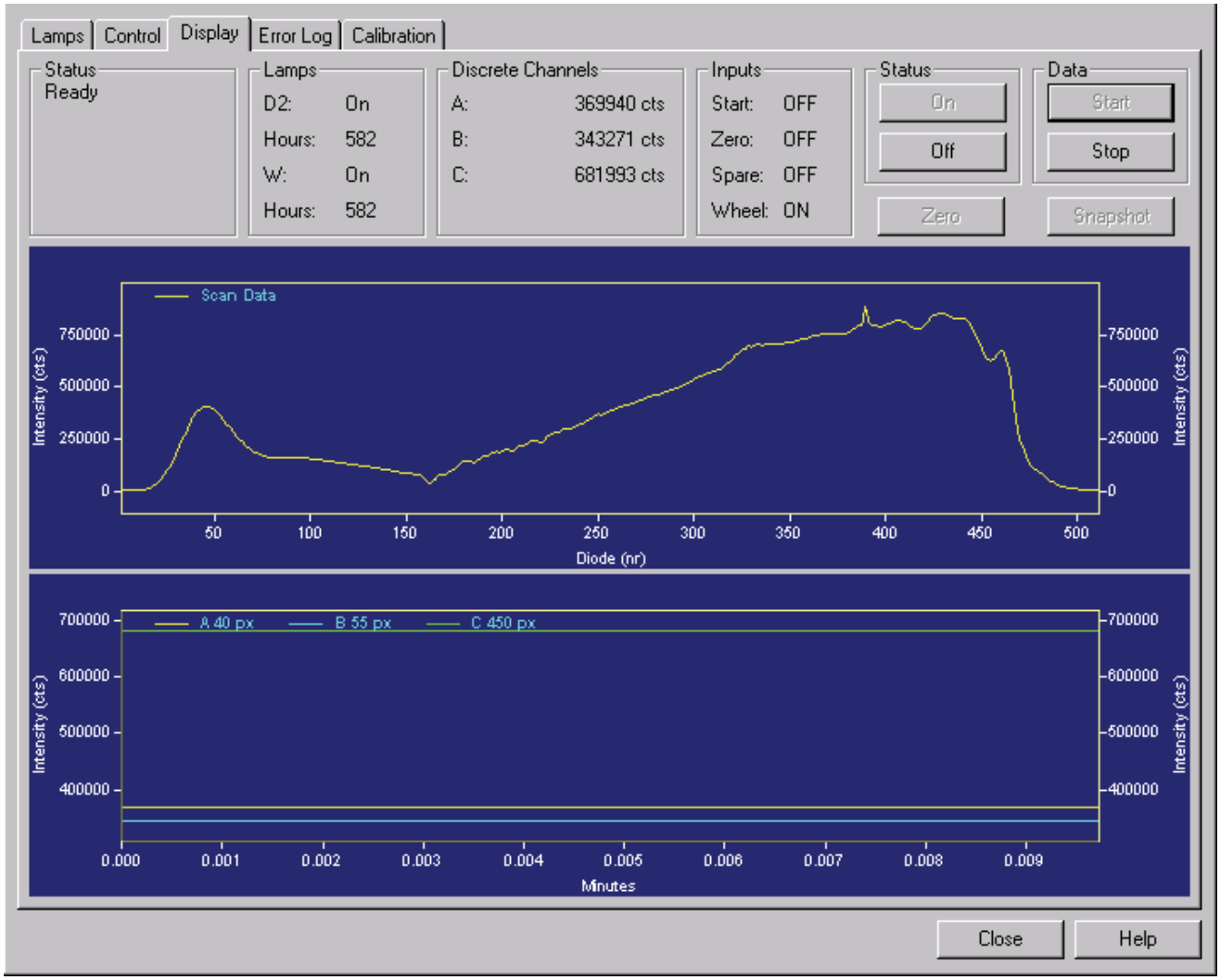
## Viewing an Intensity Scan of Both Lamps Together

### ❖ To view an intensity scan of the deuterium ($D_2$) and tungsten (W) lamps

1. If they are not already On, turn on both lamps and wait 1 h for the D2 lamp to equilibrate:
   a. From the online Instrument window, choose **Control > Instrument Status** to display the Instrument Status window.
   b. Click the Surveyor PDA Plus tab to display the Surveyor PDA Plus page. See Figure 212.
   c. Click the D2 (deuterium) Lamp On button and the W (tungsten) Lamp On button.
   d. Note the status and usage for each lamp.
2. Click **Diagnostics** to open the Surveyor PDA Diagnostics dialog box.
3. Open the Control page by clicking the Control tab. Then, set the parameters for viewing an intensity scan for the both lamps:
   a. Click the **Intensity** option in the Mode area to set the units to Intensity.
   b. Click **Default**.
   c. In the Discrete Channels area, set Channels A, B, and C to monitor diodes 94, 177, and 260, respectively.
   d. Click **Load To Detector**.

   **Note** These diodes roughly correspond to the wavelengths of 300 nm, 400 nm, and 500 nm, respectively. The Discrete Channels area on the Display page contains a digital readout for the selected diodes. See Figure 221. Record these readout values to track the performance of the deuterium lamp. You can also select other diode values to track the performance of the lamps .
4. Open the Display page, and click **Start** to refresh the display. See Figure 221.
5. Save a printout or an electronic copy of the spectrum. Date the printout and add it to your maintenance records. See "Recording the Performance of the Lamps" on page 249.

**Figure 221.** Diagnostics dialog box – Display page, showing an intensity scan with both lamps



## Viewing an Intensity Scan of the Deuterium Lamp

### ❖ To view an intensity scan of the deuterium ($D_2$) lamp

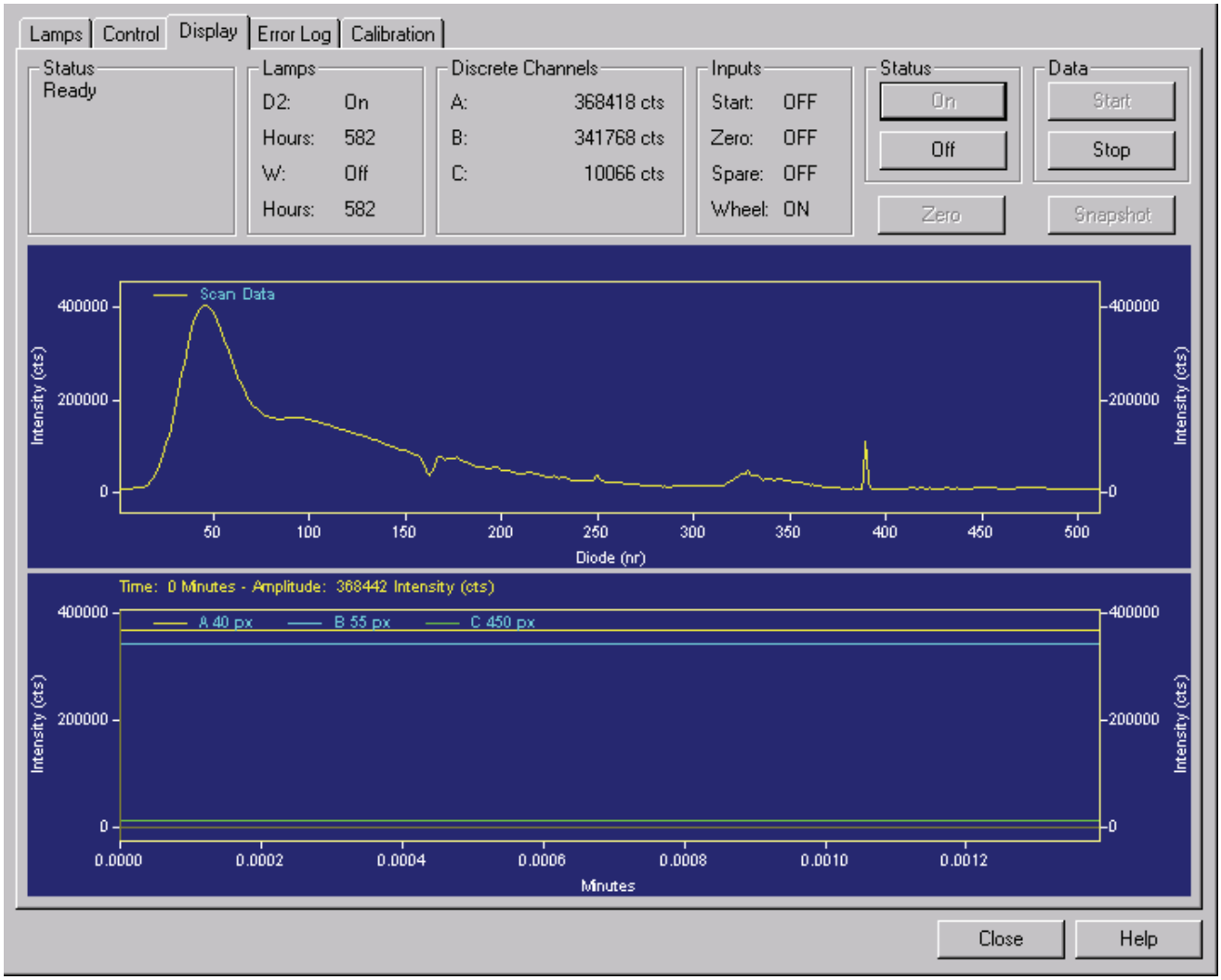
1. If it is not already On, turn on the deuterium lamp and wait 1 hour for it to equilibrate:
   a. From the online Instrument window, choose **Control > Instrument Status** to display the Instrument Status window.
   b. Click the **Surveyor PDA Plus** tab to display the Surveyor PDA Plus page. See Figure 212.
   c. Click the D2 (deuterium) Lamp On button.
   d. Note the status and usage for the deuterium lamp.
2. Ensure that the W (tungsten) lamp is Off.
3. Click **Diagnostics** to open the Surveyor PDA Plus Diagnostics dialog box.
4. Open the Control page by clicking the Control tab. Then, set the parameters for viewing an intensity scan for the deuterium lamp:
   a. Click the **Intensity** option in the Mode area to set the units to Intensity.
   b. Click **Default**.
   c. In the Discrete Channels area, set Channels A, B, and C to monitor diodes 35, 52, and 77 respectively. See Figure 222.

      **Note** These diodes roughly correspond to the wavelengths of 230 nm, 250 nm, and 280 nm, respectively. The Discrete Channels area on the Display page contains a digital readout for the selected diodes. See Figure 222. Record these readout values to track the performance of the deuterium lamp. You can also select other diode values to track the performance of the deuterium lamp.

   d. Click **Load To Detector**.

      This will allow you to view the emission intensity spectrum of the deuterium lamp.
5. Open the Display page and click **Start** in the Data area to refresh the display. See Figure 222.
6. Save a printout or an electronic copy of the spectrum. Date the printout and add it to your maintenance records. Compare this scan with similar scans that you obtain in the future to monitor any degradation in light intensity. See "Recording the Performance of the Lamps" on page 249.

**Figure 222.** Diagnostics dialog box – Display page, showing an intensity scan with the deuterium lamp



## Viewing an Intensity Scan of the Tungsten Lamp

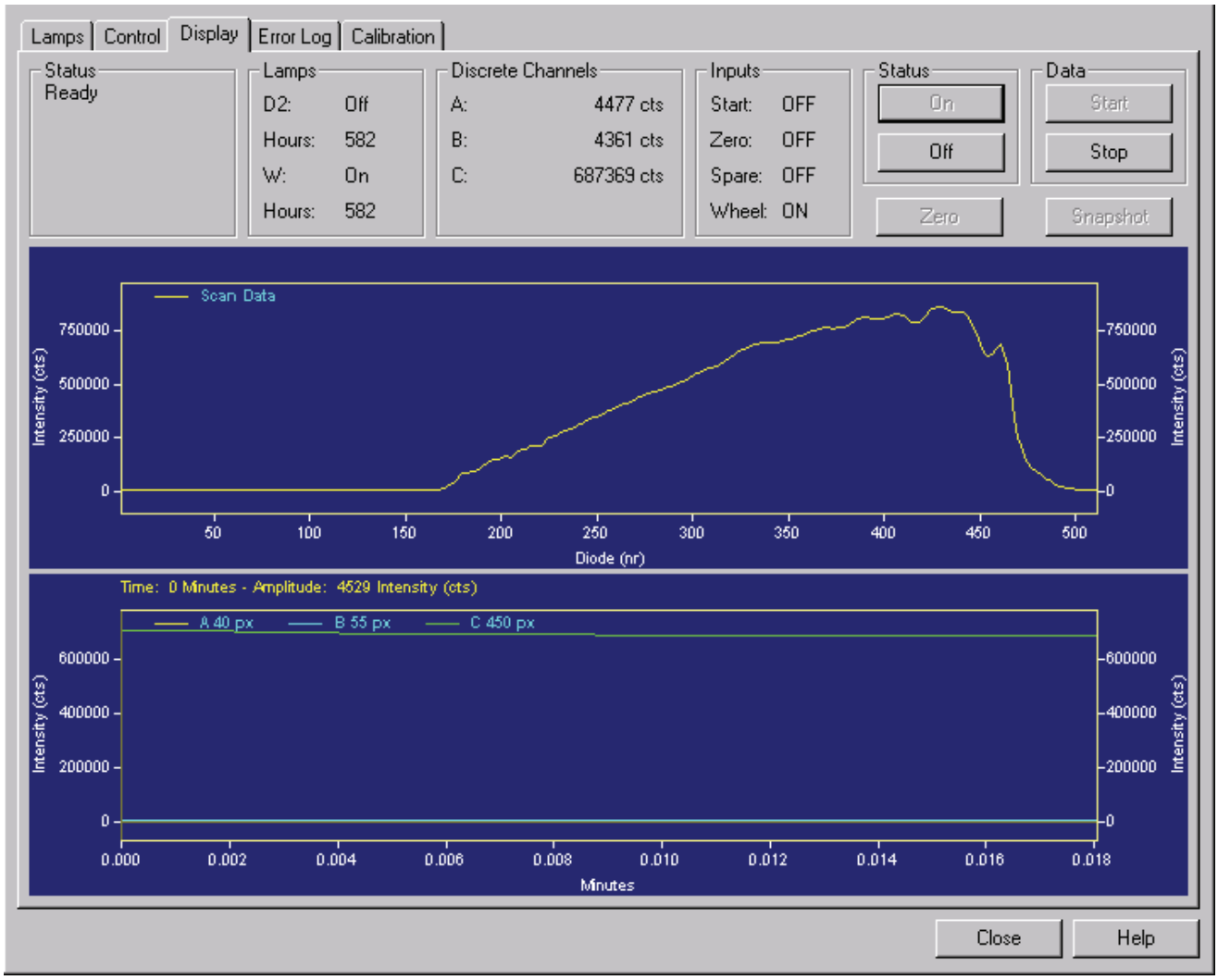
### ❖ To view an intensity scan of the tungsten (W) lamp

1. If it is not already On, turn on the Tungsten lamp:
   a. From the online Instrument window, choose **Control > Instrument Status** to display the Instrument Status window.
   b. Click the **Surveyor PDA Plus** tab to display the Surveyor PDA Plus page. See Figure 212.
   c. Click the W (Tungsten) Lamp On button.
   d. Note the status and usage for the Tungsten lamp.
2. Ensure that the D2 (deuterium) lamp is Off.
3. Click **Diagnostics** to open the Surveyor PDA Plus Diagnostics dialog box.
4. Open the Control page and set the parameters for viewing an intensity scan for the tungsten lamp:
   a. Click the **Intensity** option in the Mode area to set the units to Intensity.
   b. Click **Default**.
   c. In the Discrete Channels area, set Channels A, B, and C to monitor diodes 219, 302, and 427, respectively.
   d. Click **Load To Detector**.

   **Note** These diodes roughly correspond to the wavelengths of 450 nm, 550 nm, and 699 nm, respectively. The Discrete Channels area on the Display page contains a digital readout for the selected diodes. See Figure 223. Record these readout values to track the performance of the deuterium lamp. You can also select other diode values to track the performance of the tungsten lamp.

   e. Open the Display page and click **Start** to refresh the display.
5. Save a copy of the scan for your maintenance records. See Figure 223.
6. Turn on the deuterium lamp again and allow sufficient warm-up time before you begin acquiring data.

**Figure 223.** Diagnostics dialog box – Display page, showing an intensity scan for the tungsten lamp



## Recording the Performance of the Lamps

There are several methods available to you to record the spectrum data on the Display page in ChromQuest.

### ❖ To store the spectral data by using the print screen key

1. As you are collecting the data stream in the Display page, press SHIFT+PRT SCR.
2. Open Microsoft Paint and save the screen capture as a bitmap or open Microsoft Word and paste the screen capture into a Word document.

ChromQuest contains a print utility that allows you to print a copy of the Spectrum window.

### ❖ To use the print utility

1. In the Display page, place the cursor in the Spectrum window.
2. Right-click to open the shortcut menu.
3. Choose **Utilities > Print.**

The snapshot option allows you to create a Microsoft Excel Comma Separated Values file that contains information about the spectrum in the Display page. This file, which is overwritten each time you click the Snapshot button, is stored in the ChromQuest directory and is named WaveData.csv. The file contains three columns: diode number, wavelength, and intensity value.

### ❖ To take a snapshot

1. In the Display page, stop the data stream by clicking **Stop** in the Data area.
2. Click **Snapshot**.
3. Using Explorer, browse to the ChromQuest directory.
4. Click the WaveData.csv file. Microsoft Excel opens.
5. In Microsoft Excel, save the file with an appropriate name.

# Calibrating the Autosampler

There are four calibration options for the Surveyor Autosampler Plus: column oven calibration, tray calibration, arm calibration, and bottom distance calibration.

If you are using custom vials or custom microplates, you must perform the bottom distance calibration. The temperature calibration procedures for the column oven and tray compartments are typically performed by a Thermo Fisher Scientific service representative. If you want to perform these temperature calibrations, you must order the field service calibration tool kit which contains a customized temperature probe for the Surveyor Autosampler Plus. The XYZ arm calibration is best performed at the factory and is therefore not described in this section.

This section contains the following topics, which describe your calibration options:

- Column Oven Calibration
- Tray Calibration
- Bottom Distance Calibration

## Column Oven Calibration

The column oven calibration wizard is used calibrate the column oven temperature by using an external temperature sensor.

To perform this calibration, you must install the Oven Sensor Test Fixture that is contained in the field service calibration tool kit, and then open the Column Oven Calibration wizard in ChromQuest.

**Note** The calibration of the temperature sensor for the column oven is performed by a Thermo Fisher Scientific service representative. To perform this calibration yourself, you need to order the Field Service Calibration Kit (P/N 60053-62001).

This topic contains the following procedures:

- Installing the Oven Sensor Test Fixture
- Performing a Column Oven Calibration

## Installing the Oven Sensor Test Fixture

### ❖ To install the Oven Sensor Test Fixture

1. Open the oven door, and then loosen the top thumbscrew that holds the column clamp.
2. With the sensor facing down, slide the metal cable protector under the right side of the clamp.
3. Verify that the sensor is between the upper and lower column clamps and that it is not touching any metal.
4. Tighten the thumbscrew on the sensor, route the cable of the sensor so as not to interfere with the door, and then close the column oven door.

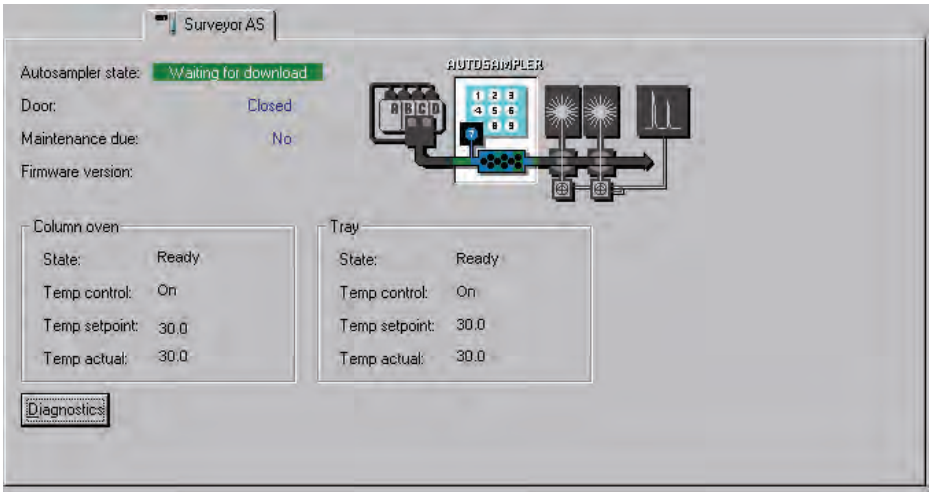
## Performing a Column Oven Calibration

Before you open the Column Oven Calibration Wizard, install the Oven Sensor Test Fixture as described above in "Installing the Oven Sensor Test Fixture" on page 251.

### ❖ To calibrate your column oven

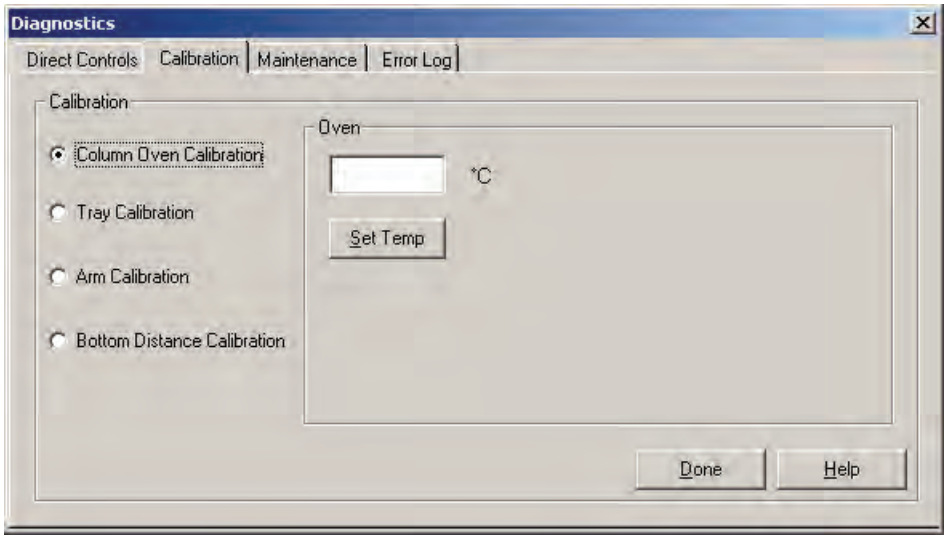
1. Open the Column Oven Calibration Wizard:
   a. From the Instrument window, choose **Control > Instrument Status**.
   b. Click the **Surveyor AS** tab to display the Surveyor AS page. See Figure 224.

   **Figure 224.** Instrument Status window – Surveyor AS page




   c. Click **Diagnostics** to open the Diagnostics dialog box.
   d. Click the **Calibration** tab, and then click the **Column Oven Calibration** option to open the first page of the Column Oven Calibration wizard, shown in Figure 225.

**Figure 225.** Calibration page of the Diagnostics dialog box with the Column Oven Calibration option selected (page 1)

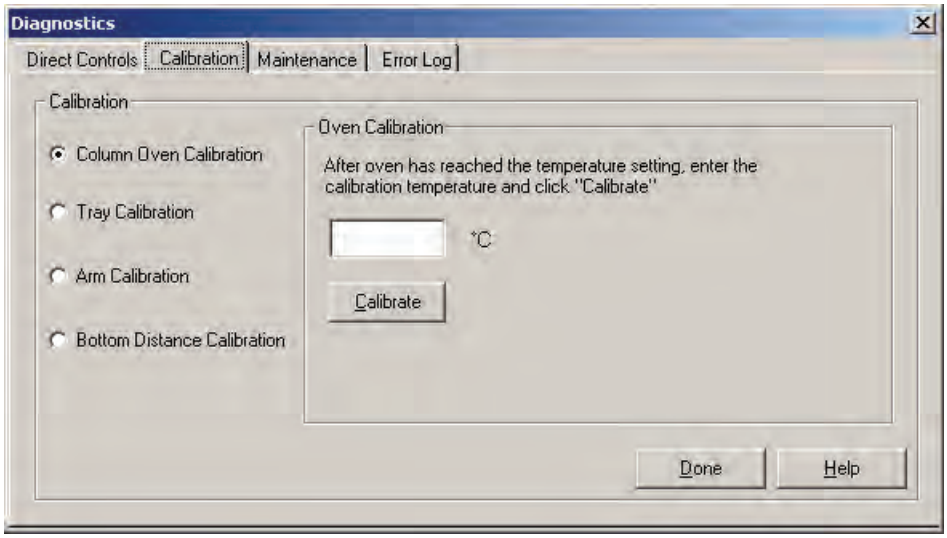



2. Size the Diagnostics dialog box so that you can see the Temp actual readout in the Column Oven area of the Instrument Status window – Surveyor Autosampler page (see Figure 224).

3. In the Oven area of the Column Oven Calibration wizard, type **30** in the box, and then click **Set Temp**.

   The second page of the Column Oven Calibration wizard appears. See Figure 226.

   **Figure 226.** Column Oven Calibration Wizard - page 2




4. In the Instrument Status window – Surveyor AS page – Column Oven area, verify that the Temp actual readout is moving towards the set point of 30.0 °C (see Figure 224).

5. When the Temp actual readout reaches exactly 30.0 °C, type the reading from the 869C thermometer in the Oven Calibration box of the Column Oven Calibration wizard (see Figure 226), and then click **Calibrate**.

   Repeat steps 4 and 5 until the readout on the 869C thermometer and the Temp actual readout in the Column Oven area of the Instrument Status window – Surveyor AS are in agreement (± 0.2 °C).

6. After the Temp actual readout and the 869C thermometer reach agreement, click **Done** to exit the Column Oven Calibration wizard.

# Tray Calibration

The Tray Calibration wizard is used to calibrate the temperature of the tray compartment by using an external temperature sensor. The Field Service Calibration kit (P/N 60053-62001) that you need to perform this calibration includes the following items:

- Calibrated Omega 869C RTD thermometer
- Surveyor A/S Vial Tray Sensor

**Note** The calibration of the tray temperature compartment is performed by a Thermo Fisher Scientific service representative. To perform this calibration, you need to order the Field Service Calibration Kit (P/N 60053-62001).

This topic contains the following procedures:

- Installing the Tray Temperature Sensor
- Performing a Tray Calibration

## Installing the Tray Temperature Sensor

### ❖ To install the tray temperature sensor

1. Open the door to the tray compartment.
2. Install the tray temperature sensor, which is a standard tray with a temperature sensor potted in a middle vial location of the tray, into location E of the tray compartment.
3. Route the cable of the sensor through the notch at the top on the tray compartment so that it does not interfere with the door closure.
4. Close the door to the tray compartment.

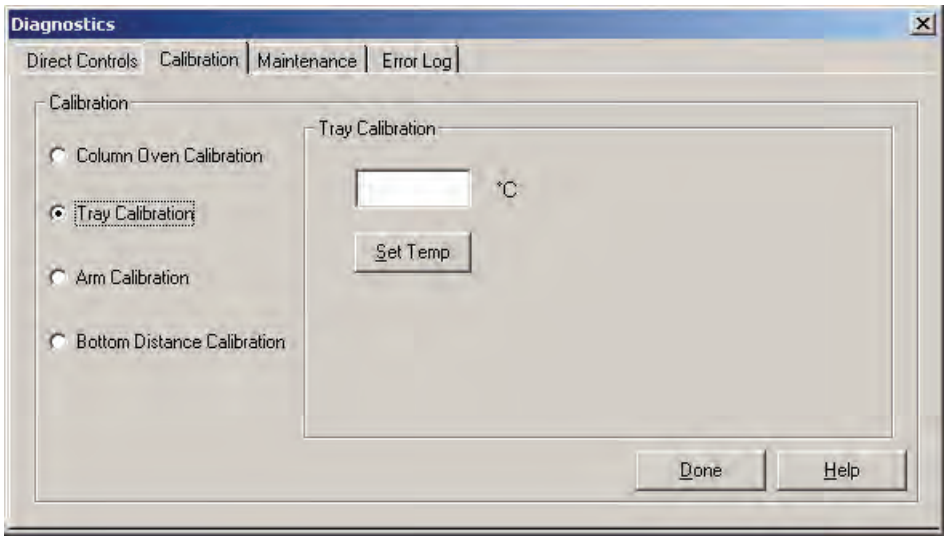
## Performing a Tray Calibration

Before you open the Tray Calibration Wizard, install the Tray Temperature Test Fixture as described above in "Installing the Tray Temperature Sensor" on page 253.

### ❖ To perform a tray calibration

1. Open the Tray Calibration wizard:
   a. From the Instrument Setup window, choose **Control > Instrument Status** to open the Instrument Status window.
   b. Click the **Surveyor AS** tab to open the status page for the Surveyor Autosampler.
   c. Click **Diagnostics** at the bottom of the Instrument Status – Surveyor AS page to open the Diagnostics dialog box for the Surveyor Autosampler.
   d. Click the **Calibration** tab to open the Calibration page.
   e. Click the **Tray Calibration** option to open the first page of the Tray Calibration wizard, shown in Figure 227.

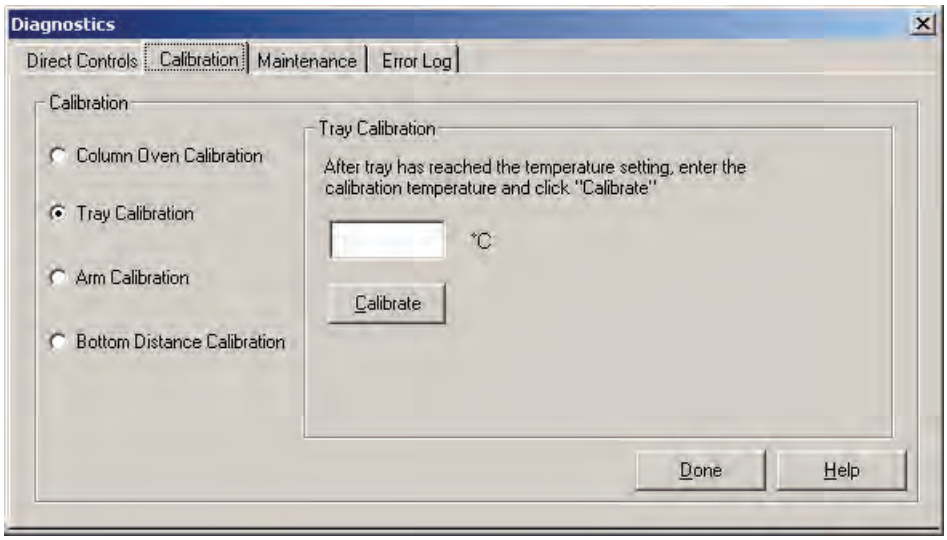
**Figure 227.** Tray Calibration wizard – page one




2. Size the Diagnostics dialog box so that you can see the Temp actual readout in the Instrument Status window – Surveyor AS page (see Figure 224).
3. In the Tray Calibration wizard – Tray Calibration area, type a value of **30** in the box, and then click the Set Temp button.

   The second page of the Tray Calibration wizard, shown in Figure 228 appears.

**Figure 228.** Tray Calibration wizard – page two




4. In the Tray area of the Instrument Status window – Surveyor AS page, verify that the Temp actual readout is moving towards the set point of 30.0 °C. See Figure 224.
5. When the Temp actual readout reaches exactly 30.0 °C, type the reading from the 869C RTD thermometer in the Tray Calibration box, and then click **Calibrate**.
6. Repeat step 4 and step 5 until the readout on the 869C RTD thermometer and the current temperature readout in the Tray area are in agreement (± 0.2 °C)
7. After the Temp actual readout in the Tray area of the Instrument Status window – Surveyor AS page and the 869C RTD thermometer reach agreement, click **Done** to exit the Tray Calibration wizard.

## Bottom Distance Calibration

You use the Bottom Distance wizard to calibrate the distance that the needle must travel to reach the bottom of a vial or well. The XYZ arm uses this value when you select one of the custom tray configurations.

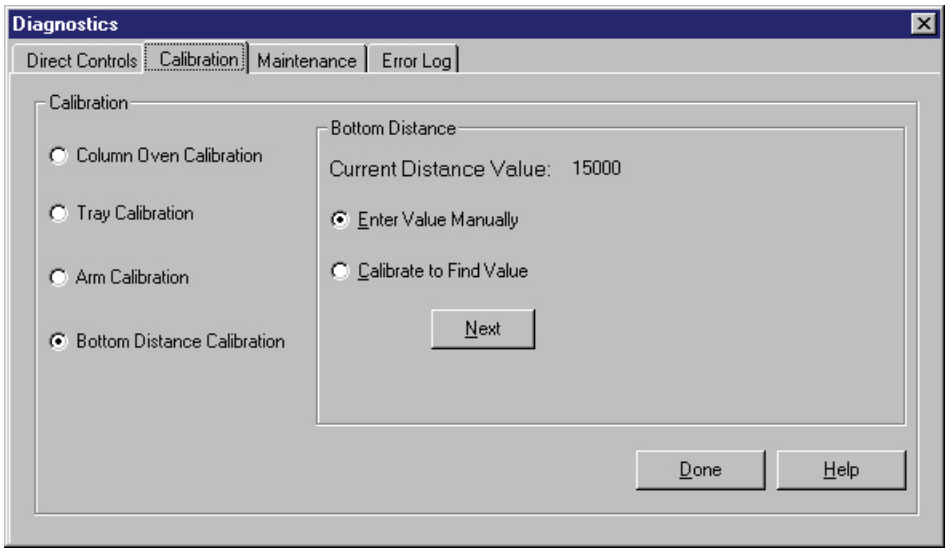
**Note** Because the autosampler stores only one value for the custom well bottom distance, you must perform the bottom distance calibration procedure each time you select a new custom tray type configuration and each time you use a different type of custom vial or custom microplate.

### ❖ To perform a bottom distance calibration

1. Open the Bottom Distance Calibration wizard:
   a. In the Surveyor AS – Diagnostics dialog box, click the **Calibration** tab to open the Calibration page.
   b. Click the **Bottom Distance** option to open the first page of the Bottom Distance wizard. See Figure 229.

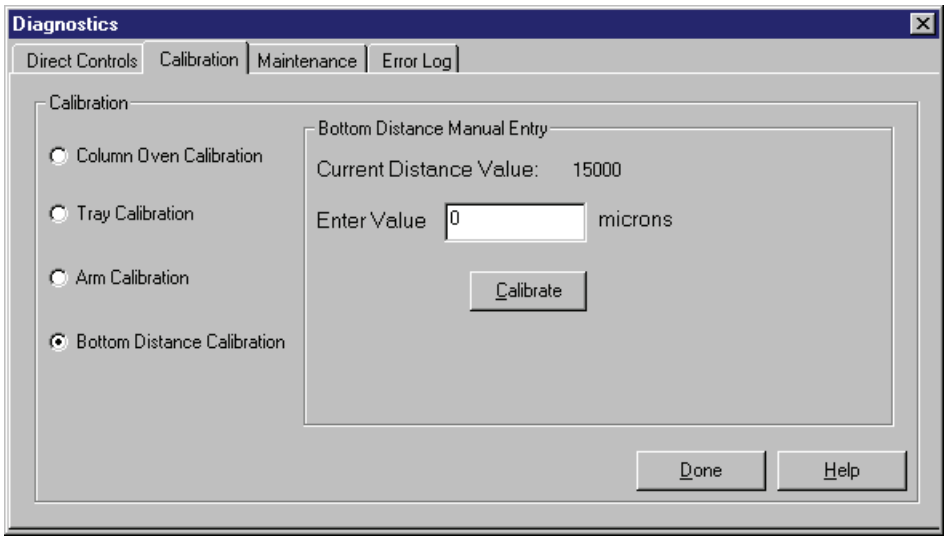
**Note** The Surveyor Autosampler stores only one bottom distance value for custom tray configurations. The number at the top of this dialog box is the current value for the distance.

**Figure 229.** Bottom Distance Calibration wizard – page one




2. Do one of the following:
   - To enter a previously determined value for the bottom distance, continue at step 3.
   - To perform an active calibration using the custom vial or well, continue at step 4.
3. To enter a previously determined value for the bottom distance:
   a. Click the **Enter the Value Manually** option (see Figure 229), and then click **Next**.
      The Bottom Distance Manual Entry area, shown in Figure 230, appears.
   b. Type in a value in microns in the Enter Value box.
      The allowable values are 15000 to 46990 (15 mm to 46.99 mm)
   c. Click **Calibrate** in the Bottom Distance Manual Entry area to upload this value into the persistent memory of the autosampler.
      The autosampler will use this bottom distance value for all custom configurations.
   d. Click **Done** to exit the Bottom Distance Calibration wizard.

**Figure 230.** Calibration page, showing the Bottom Distance Manual Entry pane




4. To perform an active calibration:

   a. Click the **Calibrate to Find Value** option to perform an active calibration, and then click **Next**.

      The Bottom Distance pane, shown in Figure 231, appears.

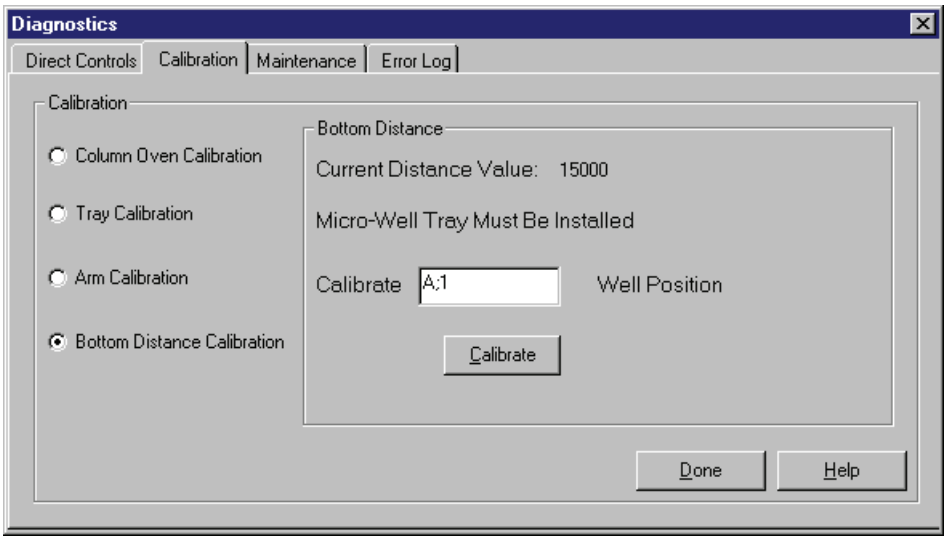
      **CAUTION** Remove vial caps or microtitre plate lids before performing an active bottom distance calibration. As the needle pierces a vial cap or a microtitre plate lid, the spring in the needle mechanism is compressed, which can cause premature activation of the needle sensor.

   b. Type a vial or well position in the Calibrate box, and then click **Calibrate**.

      The XYZ arm moves to the selected vial or well position. The needle descends until it senses the bottom of the vial or well. After the autosampler has determined the bottom distance of your selection, the current distance value appears in the Bottom Distance pane.

   c. Click **Done** to exit the Bottom Distance Calibration wizard.

**Figure 231.** Calibration page, showing Bottom Distance (active calibration) pane




# Calibrating the LC Pump

There are two calibration options for the Surveyor LC Pump Plus that affect its performance. These are the alpha setting, which affects the flow rate, and the compressibility setting, which affects the pressure pulsation of the system. The compressibility setting is set to that for water, and then the alpha setting is factory calibrated to produce an accurate flow rate for water.

If you are pumping solvents other than water and your application is sensitive to pressure pulsation, you might want to optimize the compressibility setting. Changing the compressibility setting will affect the accuracy of the flow rate. Therefore, after you optimize the compressibility setting to minimize the pressure pulsation of your system, you must check the accuracy of the flow rate. If adjusting the compressibility setting has affected the accuracy of your flow rate, you must adjust the alpha setting.

In addition to the alpha setting and the compressibility setting, the pressure transducer that is attached to the back of the purge manifold assembly is also factory calibrated. If you replace the pressure transducer, you will need to update the pressure sensor adjustment setting. Even if you never need to replace the pressure sensor, its readout tends to drift under normal usage. Therefore, you need to occasionally re-zero its output.

This topic contains the following subtopics:

# Calibration Options

The Surveyor LC Pump Plus has the following calibration options:

- Compressibility
- Pressure Recorder Full Scale
- Flow Rate Adjustment (Alpha)
- Pressure Sensor Adjustment
- Pressure Transducer Zero

## Compressibility

The compressibility of a liquid is a measure of its resistance to a decrease in volume caused by an increase in pressure. Compared to gases, most liquids are relatively incompressible. Water, a commonly used mobile phase solvent, is even less compressible than most organic solvents because of its extensive hydrogen bonding and cluster structure.

### Effect of the Compressibility Setting

Even though liquids are relatively incompressible, the compressibility of your mobile phase can affect your chromatography if you are running the LC pump at the upper limit of its pressure range. The compressibility setting of the Surveyor LC Pump Plus allows you to compensate for the compressibility of your mobile phase. Changing the compressibility setting simultaneously affects the pressure pulsation of the pump and flow rate of the mobile phase.

The compressibility effect is caused by the portion of the cam cycle in which the pistons are compressing the mobile phase. The compressibility portion of the cam cycle extends from the time at which the primary piston starts discharging until the time at which the secondary piston reaches full intake—approximately 80° of the cam cycle.

To minimize the pressure pulsation and maintain a constant flow rate, the on-board CPU compensates for this compression by making fine adjustments to the speed of the stepping motor. If the compressibility setting is incorrect, the LC pump will not be able to effectively minimize the pressure pulsation.

Figure 232 shows the effect of the compressibility setting on the pressure pulsation. The pressure trace was recorded as water was pumped at a flow rate of 3 mL/min. Three runs were recorded and the compressibility setting was changed between the runs. The compressibility settings for the three runs were 0.45 GPa-1, 1.25 GPa-1, and 3.0 GPa-1.

**Figure 232.** Overlaid LC pump pressure traces, showing the effect of the compressibility setting

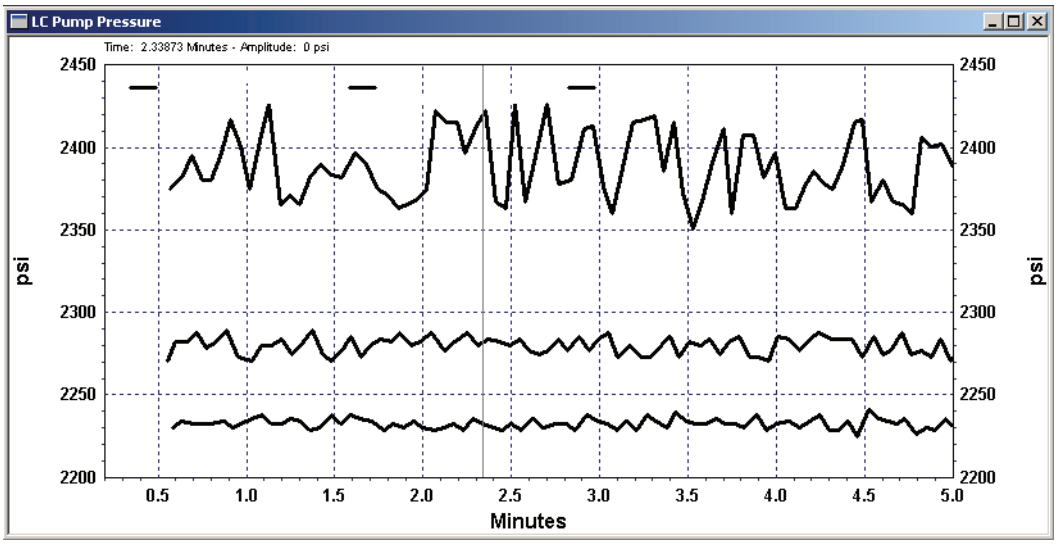



Table 12 shows a comparison of water pumped at three different compressibility settings. The correct compressibility setting for water is 0.45 GPa-1. The pressure pulsation as well as the actual flow rate increased as the compressibility setting increased.

**Table 12.** Measured flow rate and pressure pulsation vs. compressibility setting

| Compressibility Setting | %Pressure Pulsation | Actual Flow Rate (mL/min) | % Rel Diff |
|---|---|---|---|
| 0.45 $GPa^{-1}$ | ± 0.2% | 3.01 | + 0.3% |
| 1.25 $GPa^{-1}$ | ± 0.4% | 3.05 | + 1.8% |
| 3.0 $GPa^{-1}$ | ± 1% | 3.18 | + 5.9% |

### Compressibility Ratios for Common Solvents

Most mobile phases are made up of a mixture of solvents. Therefore, you need to determine the correct compressibility setting for a given mobile phase empirically. Table 13 lists the compressibility ratios for a few common solvents.

**Table 13.** Compressibility ratios for common solvents

| Solvent | Compressibility Ratio ($GPa^{-1}$) |
|---|---|
| Water | 0.45 |
| Acetonitrile | 1.20 |
| Methanol | 1.25 |
| Hexane | 1.60 |

## Pressure Recorder Full Scale

The pressure recorder full-scale calibration option allows you to set the scaling factor for the pressure trace if you choose to monitor the pressure with a chart recorder. The pressure recorder terminals are located on the back of the Surveyor LC Pump Plus. See Figure 233. The allowable range is integer values from 1 to 10.

Full scale = Set value × 4.9 MPa (50 kgf/cm2) (full scale voltage = 1 mV)

Examples:

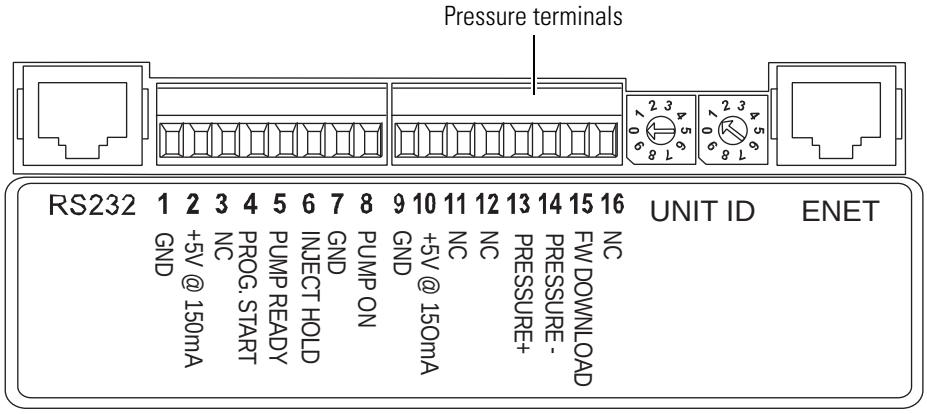
When the set value = 1, the full scale is 4.9 MPa (50 kgf/cm2)

When the set value = 10, the full scale is 49.0 MPa (500 kgf/cm2)

4.9 MPa = 50 kgf/cm2 = 49 bar = 711 psi.

**Note** This parameter is not backed up when the power is turned off. Remove vial caps or microtitre plate lids before performing an active bottom distance calibration. As the needle pierces a vial cap or a microtitre plate lid, the spring in the needle mechanism is compressed, which can cause premature activation of the needle sensor.

**Figure 233.** Terminals located on the back of the Surveyor LC Pump Plus




## Flow Rate Adjustment (Alpha)

The alpha value for your Surveyor LC Pump Plus is factory calibrated based on a flow rate of 1.000 mL/min for distilled water. A sticker containing the factory calibration value is located inside the pump on the motor casing. You can optimize the alpha value for your application by updating the alpha setting. The allowable range for alpha values is 1 to 9.9. If you are pumping water, decreasing the alpha value by 1, decreases the flow rate by 1%. Whereas, increasing the alpha value by 1, increases the flow rate by 1%.

### Pressure Sensor Adjustment

Reset this parameter when the pressure sensor is replaced. Each pressure sensor is labeled with a data sticker that contains a "0.XXXX" value for the pressure sensor adjustment setting. Multiply this value by 10,000 and then update the pressure sensor adjustment setting for your Surveyor LC Pump Plus.

### Pressure Transducer Zero

The pressure readout for the Surveyor LC Pump Plus is produced by a cell type potentiometer. This type of device tends to drift by small increments. Therefore, you will occasionally need to re-zero the readout. To update the pressure transducer zero value, turn off the pump flow and open the drain valve knob before clicking the pressure transducer zero button in the software program that operates your pump.

## Calibration Procedures

The Calibration page contains the options for fine-tuning the performance of the LC pump.

This topic contains the following procedures:

- Opening the Calibration Page
- Updating the Compressibility Setting
- Optimizing the Compressibility Setting
- Scaling the Output of the Pressure Recorder Terminals
- Updating the Flow Rate Adjustment Setting
- Updating the Pressure Sensor Adjustment Setting
- Re-zeroing the Pressure Transducer

## Opening the Calibration Page

The LC pump calibration options are accessed through the Calibration page of the Diagnostics dialog box.

**Note** To access the Calibration page in ChromQuest, you must have Instrument administration privileges.

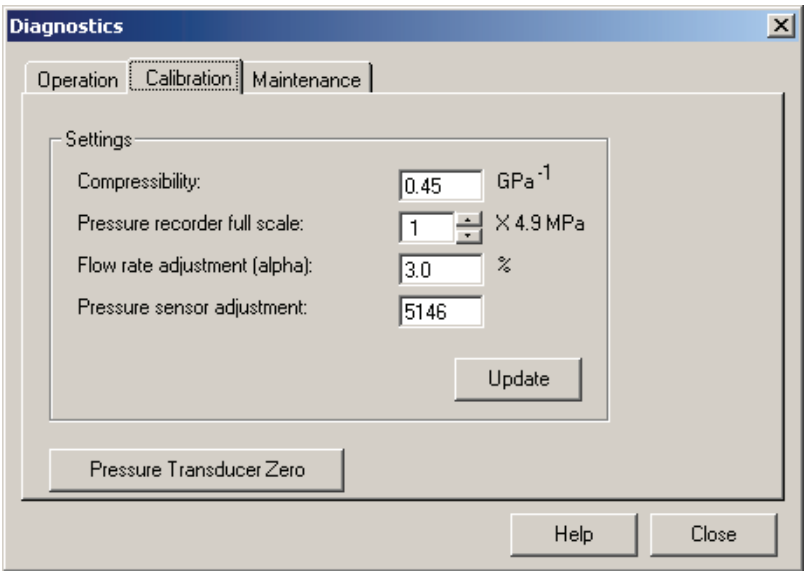
### ❖ To open the Calibration page in ChromQuest

1. Turn on the power to the pump.

2. Double-click the **ChromQuest** icon on the Windows desktop to launch ChromQuest. (The icon should be the same as that shown here.)

3. Double-click the **Instrument** button to open the online Instrument window. (The button should be the same as that shown here.)
4. In the online Instrument Window, choose **Control > Instrument Status** to open the Instrument Status window.
5. Click the **Surveyor LC Pump** tab to open the Instrument Status window – Surveyor LC Pump page.
6. Click **Diagnostics** at the bottom of the Surveyor LC Pump page to open the Diagnostics dialog box for the pump.
7. Click the **Calibration** tab to open the Calibration page, shown in Figure 234.

**Figure 234.** Surveyor LC Pump Diagnostics dialog box – Calibration page



## Updating the Compressibility Setting

If you have already determined the best compressibility setting for your application, update the compressibility setting of the LC pump.

### ❖ To update the compressibility setting in ChromQuest

1. In the Instrument window, open the Calibration page for the pump as described in "Opening the Calibration Page" on page 263.
2. Type a new value in the Compressibility setting box, and then click **Update**.

   A dialog box containing a warning message appears.
3. Click **OK** in the dialog box to update the compressibility setting.

## Optimizing the Compressibility Setting

### ❖ To optimize the compressibility setting of the LC pump

1. Add an auxiliary LC Pump pressure trace to your method:
   a. Open your method by choosing **File > Method > Open**.
   b. Click the **Auxiliary trace** tab in the Instrument Setup window.
   c. Select the LC Pump Pressure acquire check box.
   d. Save the method by choosing **File > Method > Save**.
2. Download the method that contains the pressure trace by choosing **Control > Download Method**.
3. Preview the run and print out the pressure trace:
   a. Choose **Control > Preview Run**.
   b. Choose **Window > LC Pump Pressure**.
   c. Preview the run for a few minutes, and then print out the LC pump pressure trace. To print the trace, right-click to open the Chromatogram shortcut menu and choose **Utilities > Print**.
4. Stop the preview run by clicking the **Stop**  button in the Instrument toolbar.
5. Change the compressibility setting as described in "Updating the Compressibility Setting" on page 264.
6. Repeat step 3, step 4, and step 5 until you are satisfied with the results.
7. After you determine the best compressibility setting for your application, enter the value in the Compressibility setting box and click **Update**.

**Note** Changing the compressibility setting will also affect the flow rate. After you change the compressibility setting, measure the flow rate.

## Scaling the Output of the Pressure Recorder Terminals

The pressure recorder terminals are located on the back panel of the Surveyor LC Pump Plus.

### ❖ To set the full-scale output of the pressure recorder terminals

1. Open the Calibration page for the pump as described in "Opening the Calibration Page" on page 263.
2. Type a value from 1 to 10 in the Pressure Recorder Full-Scale box, and then click **Update**.

   A dialog box containing a warning message appears.
3. In the dialog box containing the warning message, click **OK** to update to scaling output of the pressure recorder terminals.

## Updating the Flow Rate Adjustment Setting

Change the flow rate adjustment setting if the flow rate is not accurate.

### ❖ To update the flow rate adjustment setting in ChromQuest

1. Open the Calibration page for the pump as described in "Opening the Calibration Page" on page 263.
2. Enter a new value in the Flow rate adjustment (alpha) box.
3. Adjust the setting:
   - If the actual flow rate is below the set value, increase the alpha value.

     If you are pumping water, increasing the alpha value by 1, increases the flow rate by 1%. For example, if the pump is set to deliver a flow rate of 1 mL/min and the actual flow rate is 0.96 mL/min, which is 4% below the set rate, raise the current setting by 4. If the current setting is 5.0, enter a new setting of 9.0.
   - If the actual flow rate is above the set value, decrease the alpha value.

     If you are pumping water, decreasing the alpha value by 1, decreases the flow rate by 1%. For example, if the pump is set to deliver a flow rate of 1 mL/min and the actual flow rate is 1.04 mL/min, which is 4% above the set rate, lower the current setting by 4. If the current setting is 5.0, enter a new setting of 1.0.
4. Click **Update**.

   A dialog box containing a warning message appears.
5. In the dialog box containing the warning message, click **OK** to update the alpha value setting.

## Updating the Pressure Sensor Adjustment Setting

Reset this parameter when the pressure sensor is replaced. Each pressure sensor is labeled with a data sticker that contains a "0.XXXX" value for the pressure sensor adjustment setting. Multiply this value by 10,000 and enter the result in the Pressure Sensor Adjustment setting box.

### ❖ To update the pressure sensor adjustment setting

1. Open the Calibration page in ChromQuest as described in "Opening the Calibration Page" on page 263.
2. Enter the value on the data sticker "0.XXXX" multiplied by 10,000 in the Pressure Sensor Adjustment setting box.
3. Click **Update**.

   A dialog box containing a warning message appears.
4. In the dialog box containing the warning message, click **OK** to update the Pressure Sensor Adjustment setting.

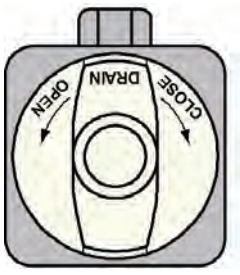
## Re-zeroing the Pressure Transducer

The offset error of the pressure transducer is the value that is displayed when under a particular set of conditions, such as the purge manifold knob being set to the open position, it should be zero. You can correct for this offset error by re-zeroing the pressure readout of the pressure transducer when the purge manifold knob is open.

### ❖ To re-zero the output of the pressure transducer from ChromQuest

1. Stop the pump flow:
   a. Choose **Control > Instrument Status > Pump tab**.
   b. Click **Stop Pump**.
2. Open the drain valve by gently turning it counterclockwise 180° to ensure that the transducer is actually sensing zero system pressure. See Figure 235.

   **Figure 235.** Drain valve in open position

   
   
3. Open the Calibration page for the pump as described in "Opening the Calibration Page" on page 263.

4. Click **Pressure Transducer Zero**.
5. When you are finished zeroing the pressure transducer, close the drain valve by gently turning it clockwise until you feel resistance.

**Note** Applying excessive force to the drain valve knob will wear out its O-ring at a faster rate.

# Calibrating the RI Detector

Validate the accuracy of the RI detector on a regularly scheduled basis by using the built-in validation procedure accessed from the ChromQuest data system.

To validate the accuracy of the detector, perform the following procedures:

1. Preparing to Perform the Validation Procedure
2. Performing the Validation Procedure
3. Restoring the RI Detector to Normal Operation

## Preparing to Perform the Validation Procedure

Before you can perform the validation procedure, you must set up your LC system to pump HPLC grade water and you must prepare a sucrose validation standard. To prepare for the validation procedure, perform the following procedures:

- Preparing the Sucrose Validation Standard
- Setting Up the LC System to Validate the RI Detector

### Preparing the Sucrose Validation Standard

You inject a 0.35% by weight sucrose in water solution as part of the validation procedure.

❖ **To prepare a fresh sucrose solution**

1. Weigh out 350 mg of sucrose, and transfer quantitatively to a 100 mL volumetric flask.
2. Add approximately 50 mL of HPLC grade water to the flask. Swirl the flask to dissolve the sucrose, and then fill the flask to volume with water.

### Setting Up the LC System to Validate the RI Detector

Before you perform the validation procedure for the RI detector, the LC system must be set up to pump HPLC grade water. In addition, it is a good practice to flush the flow cell of the RI detector with HPLC grade water.

### ❖ To set up the LC system for the validation procedure

1. Fill one of the solvent reservoir bottles with 100% HPLC grade water.
2. To purge the flow cell of the RI detector, follow the instructions in "Purging the Flow Cell of the RI Detector" on page 117.
3. Leave the program open to the RI Diagnostic page and proceed to the next procedure.

## Performing the Validation Procedure

Use the validation procedure to validate the accuracy of the RI detector and to ensure the optimal performance of the optical system.

To perform the validation procedure, you must have the following items provided in the accessory kit for the RI detector:

- 10 mL syringe
- Syringe adapter

### ❖ To validate the accuracy of the detector

1. Initiate the validation procedure:
   a. If it is not already open, open the RI Diagnostic page.
   b. In the Calibration list, select **Validation Procedure**. See Figure 236.
   c. Click **Go**.

      The popup message, shown in Figure 237, appears.

      **Figure 236.** RI Diagnostic page, showing the selection of the Validation Procedure

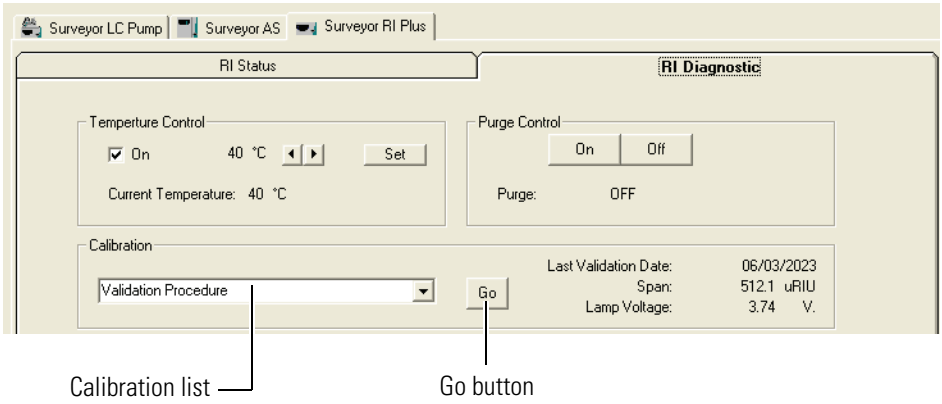
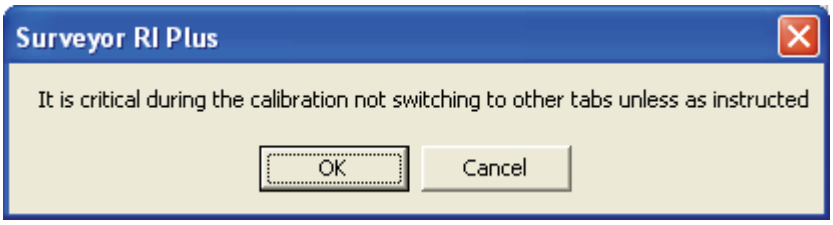


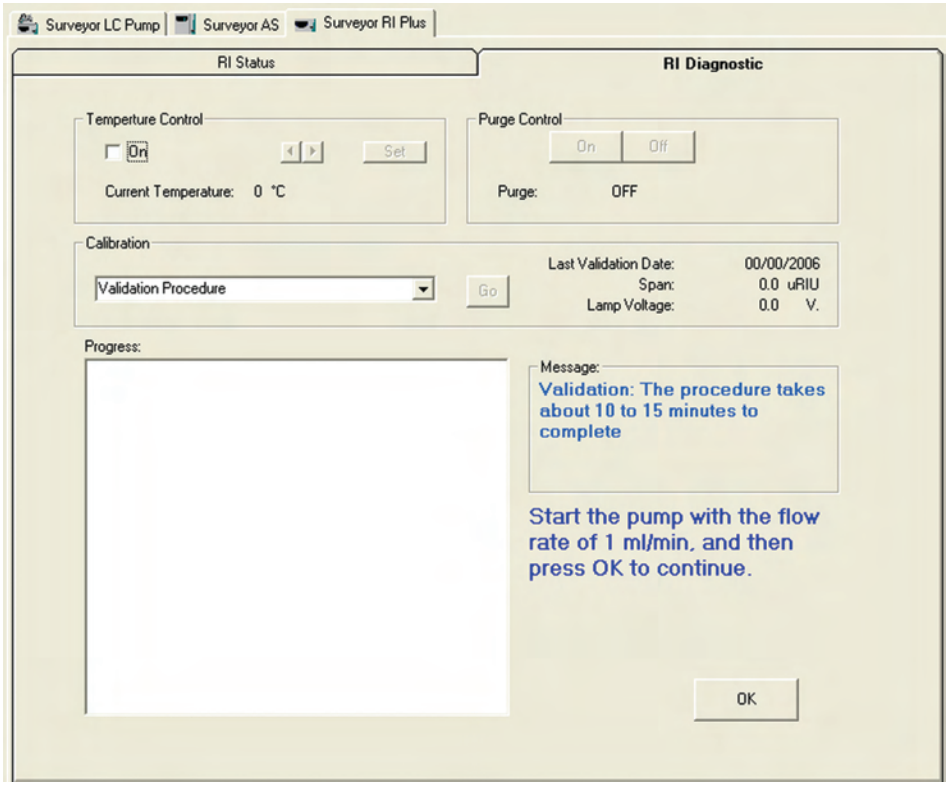
**Figure 237.** Popup message, warning you not to switch between tabs




d. Click **OK** in the popup message.

Two messages appear, as shown in Figure 238. The message in the Message box informs you how long the validation procedure takes to complete. The message below the message box instructs you to start the pump, which should be set to deliver HPLC grade water at a flow rate of 1 mL/min.

**Figure 238.** Messages that appear at the beginning of the validation procedure

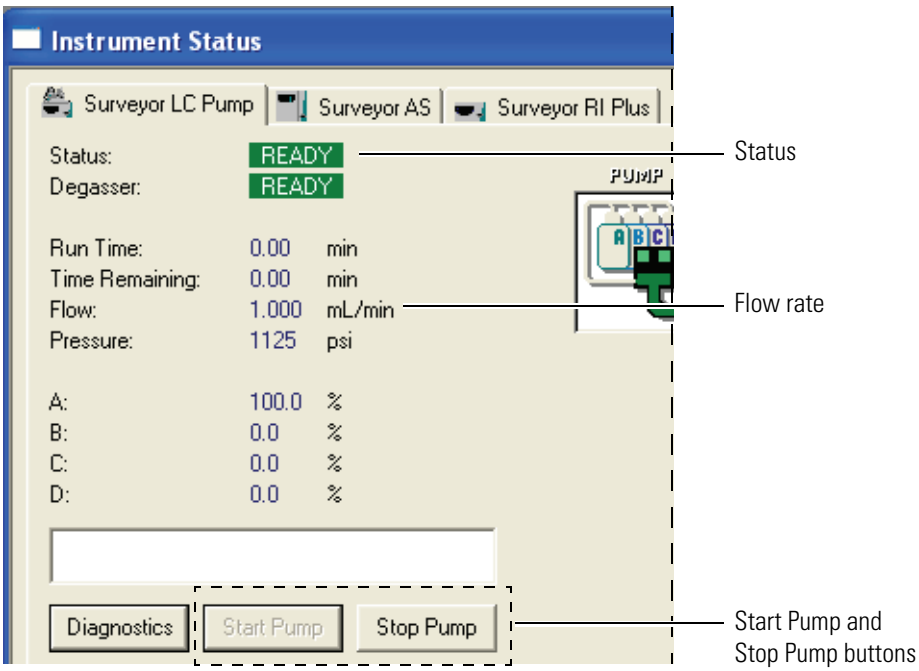



2. Do one of the following:

- If the pump flow is already on, proceed to step 4.
- If the pump flow is off, proceed to step 3:

3. To turn on the pump flow:
   a. Click the **Surveyor LC Pump** tab.
   b. Click **Start Pump**.
   c. Check that the flow rate is set to 1 mL/min and that the status of the pump is READY, as shown in Figure 239.

      **Figure 239.** Instrument Status window – Surveyor LC Pump page




   d. Return to the RI Diagnostic page by clicking the **Surveyor RI Plus** tab and the **RI Diagnostic** tab.

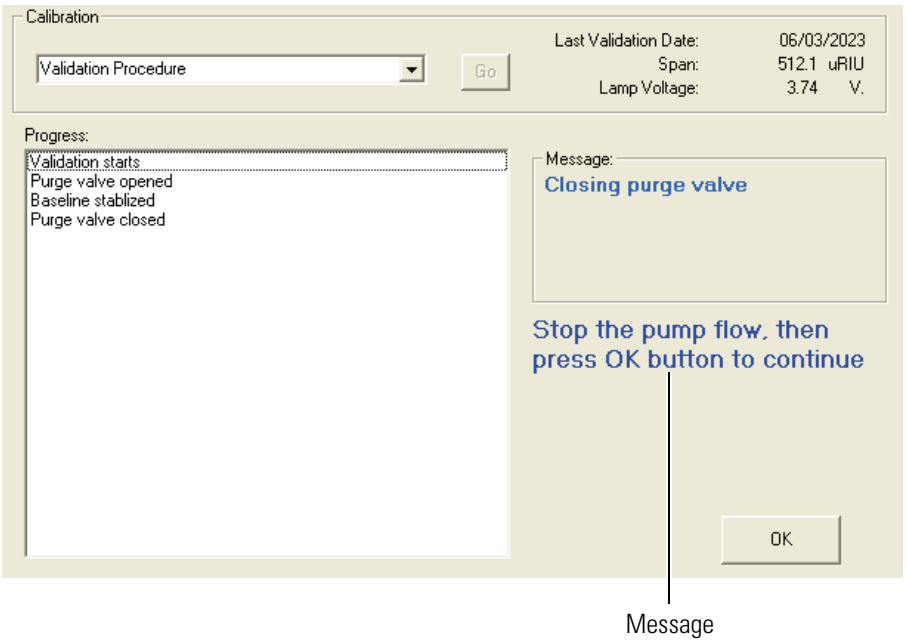
4. Click **OK** in the **RI Diagnostic** page.

   ChromQuest performs the following tasks in the order listed:

   - Sends a signal to the detector to open the purge valve.
   - Measures the baseline stability as water is pumped through both the reference and sample compartments of the flow cell.
   - Sends a signal to the detector to close the purge valve.

   After the purge valve closes, the message, shown in Figure 240, appears below the Message box. The message instructs you to stop the pump flow.

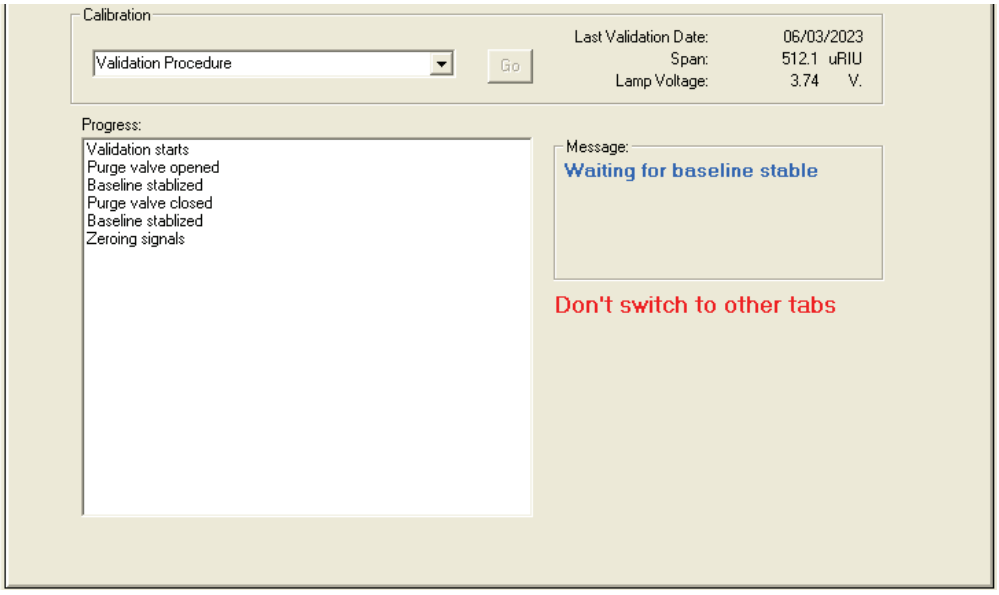
**Figure 240.** Message instructing you to turn off the pump flow




5. Click the **Surveyor LC Pump** tab, and then click **Stop Pump**.
6. Return to the RI Diagnostic page by clicking the **Surveyor RI Plus** tab and the **RI Diagnostic** tab.
7. Click **OK** below the stop pump flow message.

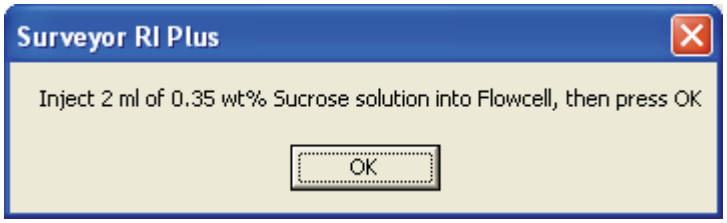
   ChromQuest performs a second baseline stability check, and then zeroes the signal from the detector. See Figure 241.

**Figure 241.** Ri Diagnostic page, showing the progress of the Validation Procedure



After ChromQuest zeroes the signal from the RI detector, the popup message, shown in Figure 242, appears.

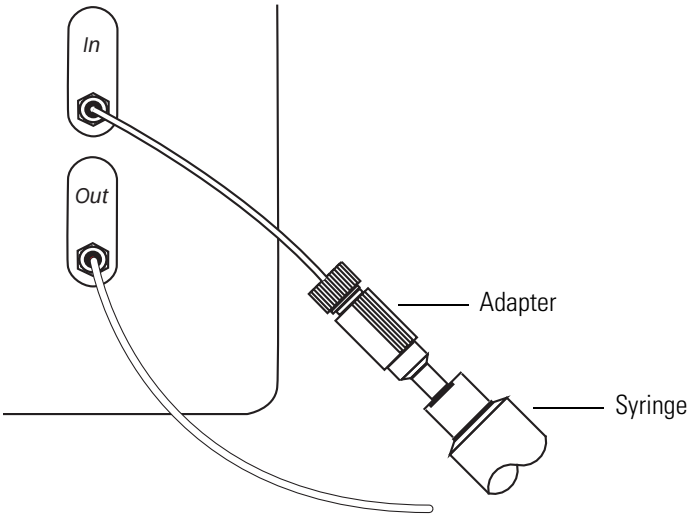
**Figure 242.** Popup message instructing you to inject the sucrose solution




8. To inject the sucrose solution, do the following:

   a. Disconnect the LC system from the In port of the RI detector, and then connect an adapter to the In port of the RI detector, as shown in Figure 243.

   **Figure 243.** Injecting the sucrose solution




**CAUTION** The flowcell cannot withstand a backpressure greater than 520 kPa (75 psi). Therefore, you can easily break the flowcell by forcing fluid through it.

   b. Fill a syringe with the freshly prepared sucrose solution, and then gently inject approximately 2 mL or less of the solution into the IN port of the detector.

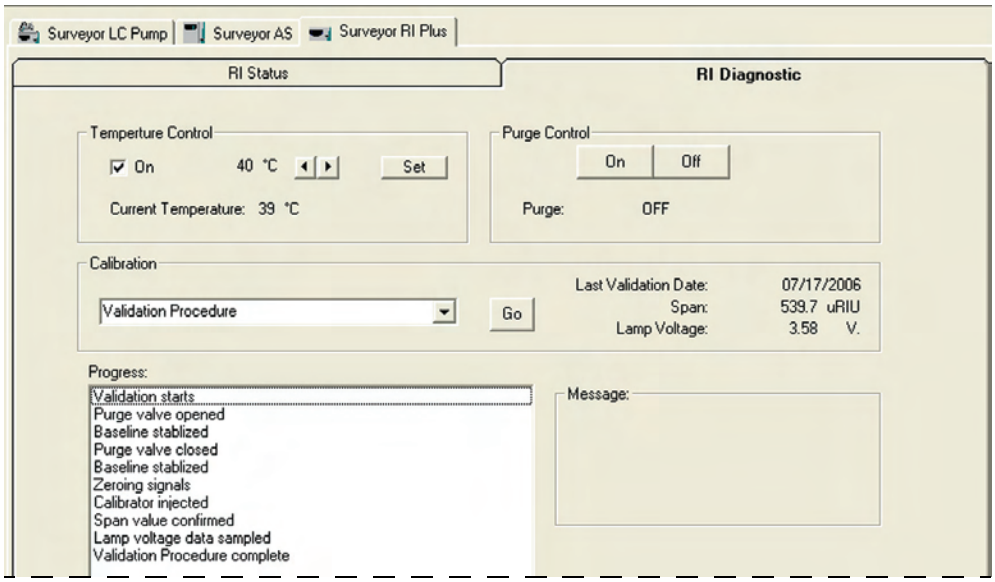
   c. In the popup message instructing you to inject the sugar solution (see Figure 242), click **OK**.

   In the Progress area, the following messages appear: Calibrator injected and Span value confirmed. The detector measures the refractive index of the sucrose solution, and ChromQuest compares the measured value to the expected value of µ512 RIU.

9. Depending on whether the span confirmation passes or an error message appears, do the following:

   - If the span confirmation passes, as shown in Figure 244, click **OK** in the popup message that appears, and then proceed to the next procedure, "Restoring the RI Detector to Normal Operation" on page 274.
   - If an error message appears, proceed to step 10.

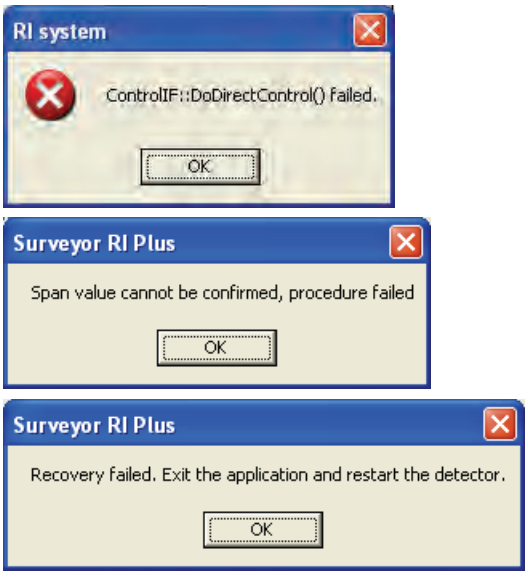
**Figure 244.** Validation procedure complete, showing the popup message




10. To recover from a span confirmation error:

    a. Click **OK** in each popup message that appears. Figure 245 shows the messages that appear.

**Figure 245.** Span confirmation failure messages



b. Turn off the power to the RI detector.

c. Exit the Instrument window.

d. Turn the power to the RI detector back on.

e. Wait until the instrument becomes available in the Main Menu window of ChromQuest, and then double-click the button for the instrument to open the online Instrument window.

f. Restart the validation procedure at step 1 on page 268.

## Restoring the RI Detector to Normal Operation

❖ **To restore detector to normal operation**

1. Remove the syringe from the IN port of the detector.
2. Re-attach the tubing to the IN port of the detector.
3. Click the **Surveyor LC Pump** tab, and then click **Start Pump**.
4. Return to the RI Diagnostic page by clicking the **Surveyor RI Plus** tab and the **RI Diagnostic** tab.
5. Under Purge Control, click **On** to rinse the sample and reference compartments of the flow cell with HPLC grade water.
6. After a period of approximately 5 to 10 minutes, click **Off** in the Purge Control area to close the purge valve and to return to normal operation.

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